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MODEL PROGRAMME FOR THE PRODUCTION OF VACCINES IN DEVELOPING COUNTRIES

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Terminal report*

Prepared by the
United Nations Industrial Development Organization

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IN MEMORIAM

TOON VAN WEZEL

This publication is dedicated to the memory of Dr. A. L. van Wezel (Toon) whose tragic death came as a profound shock to all his colleagues who worked under his inspiring guidance and leadership during the development of this Model Plan.

His contribution to this volume was considerable. UNIDO salutes a great expert and a valiant servant of humanity whose knowledge, expertise and charming manner has earned for him the undying respect and affection of all those who worked in this common endeavour.

May he rest in peace.

Explanatory Memorandum

The Model Programme for the Production of Vaccines in Developing Countries is intended primarily for use by government officials and directors of institutes who are responsible for structuring and implementing national immunization programmes. The Model programme may also be of general guidance to the professional staff members in charge of vaccine production. It may, in addition, serve as a basis for comparing different technologies applied in other laboratories for the manufacture and quality control of conventional vaccines.

This report summarises the RIVM's experience in production and control of conventional bacterial and viral vaccines currently used in national immunization programmes in the Netherlands. It therefore offers only one option among a number of technologies. The technology described is nevertheless considered to be in the fore-front of some areas of vaccine production, i.e. the unit-processing principle as proposed by van Hemert.

It must therefore be stressed that this is a Model Programme and that there are alternative production systems and methods. The production of oral poliomyelitis vaccine is not included in the present Programme; it will be covered in a later UNIDO document.

Understandably, a preliminary analysis of the local industrial infrastructure and prevailing economic conditions must be made, and appropriate scientific and technological educational programmes must be devised if the required transfer of technology is to take place successfully. A good organizational and managerial infrastructure with the optimal use of human resources is essential for the success of such undertaking. There are, of course, other aspects of technology transfer over and above the execution of a project plan derived from a sound proposal.

A continuing support services plan may be envisaged at the termination of the execution stage. Such a plan can comprise, on a yearly or two-yearly basis: a GMP audit, spare part procurement, performance of parallel quality control tests, training of new senior staff, implementation of new WHO requirements and raw material testing. Additional aspects include the guarantee that the supplier shall install the purchased equipment and ensure that all machinery performs according to specifications. Maintenance and training of maintenance personnel are key considerations. A full understanding of the concept of quality assurance is equally important. Last but not least, a long-term funding commitment is indispensable for projects involving the production and control of biological products.

The Model Programme will be followed by a series of UNIDO publications including technical documents on production and quality control of BCG vaccine produced on surface culture and of oral poliomyelitis vaccine, a paper on technology transfer for biological production and a directory of potential partners in transfer of technology for biological production.

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LIST OF ABBREVIATIONS

ATTC	American Type Culture Collection
BCG	Bacillus Calmette-Guérin
BPL	Beta-propiolacton
c.	circa
DMSO	Dimethylsulfoxid
DU	D-antigen units (polio)
ELISA	Enzyme Linked Immuno Sorbent Assay
EPI	Expanded Programme on Immunization
f. or ƒ	Dutch guilder
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
HDC	Human Diploid Cells
IOU	International Opacity Units
IU	International Units
Lf	Flocculation Units (diphtheria, tetanus)
MEM	Minimum Essential Medium
MMR	Measles, Mumps, Rubella
MW	Molecular Weight
NPV	Net Present Value
p.a.	pro analysis
PBS	Phosphate-Buffered Saline
PFU	Plaque-Forming Units
RIVM	Rijksinstituut voor Volksgezondheid en Milieuhygiene (Bilthoven)
R&D	Research and Development
$TCID_{50}$	Tissue Culture Infecting Dose
THD	Total Human Dose
UNIDO	United Nations Industrial Development Organization
WCB	Working Cell Bank
WHO	World Health Organization

GENERAL INTRODUCTION

In the meeting of February 1984 of the Advisory Panel of Preventive Medicine UNIDO it was recommended, that a study should be made on the impact of modern technologies on production during the fortcoming decade of priority biologicals (ref.1; further details and back-ground information in ref.2-6).

In the course of the meeting the Director-General of the Rijksinstituut voor Volksgezondheid en Milieuhygiene (National Institute of Public Health and Environmental Hygiene, to be indicated further in this document as RIVM) offered to provide UNIDO with confidential and non-confidential information with regard to the production of priority conventional vaccines, subject to clearance by the RIVM for the preparation of a model programme by UNIDO which could be used as a project concept and also for individual projects.

This offer was accepted by UNIDO and a group of consultants in vaccine production, quality control, engineering and economics in the RIVM were requested, to collect necessary techno-economic information for the preparation of a model programme for industrial production of biologicals in close cooperation with the UNIDO secretariat.

This model programme is intended primarily for use by Government officials and directors of institutes, responsible for framing and implementing national vaccination programmes. In addition it can be used as guidance by professional staff members responsible for the production of them. It can also serve as a base for comparison of different technologies applied in other laboratories for production and control of conventional vaccines.

In the past decades some countries in Asia and South and Central America have on the basis of existing scientific organization established regular educational programmes at university level, to create a nucleus of scientists. This has enabled them to make further considerable progress in absorbing scientific knowledge. Among those countries there are now several where capable and intelligent young investigators can follow, or themselves take part in the generation of new knowledge. The major scientific research of these Centres is at present still concentrated in the developed world and knowledge is acquired there at an increasing rate. On the other hand, in only a few developing countries is the "knowledge community" structured in such a way that new knowledge is not only absorbed in the academic centres, but also that it can be put to practical use for the benefit of the community.

This model report summarizes experience at the RIVM in production and control of conventional bacterial and viral vaccines currently used in national immunization programmes. It thus offers only one choice of a number of technologies. It is nonetheless well proven technology considered to be in the forefront in some areas of vaccine production.

For many years the production of vaccines used in national immunization programmes has been a routine process divorced from the main stream of scientific acitivities. In many countries it has been consigned to government institutes. In fact, with some notable exceptions, pharmaceutical industry has become less interested to make the large investments in research and development for vaccines. As a result, insufficient use is often made of scientific developments in the fields of biochemistry and biochemical engineering, immunology, microbiology, virology and animal husbandry. In particular, the manufacturing process is often ill defined and even obsolete. In some instances this will yield relatively crude products (an example is pertussis vaccine), and in addition there is poor consistency between consecutive production lots. In many cases the production process is still largely manual. This leads to a vicious downward spiral where low technology leads to a lack of intellectual stimulation and research. It should be emphasized that, in spite of the rather limited investments made in the development of conventional vaccines, the Expert Committee on Biological Standardization of WHO has established a number of excellent reference systems and materials, which are widely used. These systems have certainly guided many countries in the production of vaccines of acceptable quality. In the case of smallpox vaccine, the quality control programmes of WHO have contributed to the world wide eradication of this disease.

Meanwhile the rather low prices paid for vaccines have limited the number of manufacturers, in private and public sectors, interested in the production of conventional vaccines and discouraged many organizations in the developing world from establishing their own production facilities.

In recent years a number of developed countries have introduced a new form of quality control. In addition to using as reference the standards established by WHO for laboratory facilities for the control of vaccines and sera, they have instituted methods in compliance with rules for Good Manufacturing Practice (GMP) that have been formally drawn up in recent years. These rules include procedures for the qualification of personnel and their on-the-job training, the establishment and maintenance of building facilities and equipment for production, control and formulation, and the necessary control of raw materials.

GMP procedures established for the production of conventional vaccines will be generally applied to the growing field of biotechnology, regardless of whether the end product will be used in human or veterinary medicine, agriculture or in industrial processes.

This report is based on the experience at RIVM where the principle of unit processing proposed by van Hemert (10) has been adopted. The main vaccines considered in this report are all made using the same basic system employing a stainless steel bioreactor unit (the Bilthoven Unit) which can be sterilized in situ and controlled for parameters concerned with microbial cell growth. These include pH, pO_2, pCO_2; also sampling to determine growth, opacity and titre of the required biological product. The use of the Bilthoven Unit allows the same conditions to be maintained during scale-up and greatly improves the consistency of the process. In the beginning it could not be used for virus cultivation, which requires as substrate mammalian cells obtained from animal organs (kidneys), or consisting of so-called cell strains or cell lines. The microcarrier system developed subsequently by van Wezel at the RIVM made it possible to grow large quantities of mammalian cells on the surface of plastic-like particles, which could be held in suspension in the Bilthoven Unit. Large amounts of virus materials can thus be obtained, while modern purification procedures ensure the effective removal of contaminating substances yielding an end product devoid of impurities including RNA or DNA.
The use of (semi-)industrial techniques, such as the unit process for production of conventional vaccines, will no doubt be stimulated by the simultaneous introduction of GMP. In this report it will be shown how relatively small amounts (in term of human doses) of different vaccines can be prepared in consecutive production rounds with the same facilities and personnel, provided the GMP-rules are strictly observed. There appears to

be no principal objection, for instance, to using in the more distant future the same building facilities and equipment for the production of tetanus toxin and BCG vaccine.

While originally cell lines were not permitted for the cultivation of virus materials for human use (even if inactivated) , there is at present a tendency favouring their acceptance for human vaccine production.

It could be argued that the existence of facilities for the production and quality control of conventional vaccines will contribute to a number of inter-related, social and economic objectives: reduction of dependance on supplies requiring foreign currency; more efficient use of national resources; increase of domestic stocks of technical know-how and human capital in many disciplines used in development of biologicals and creation of employment.

As was remarked at the beginning of this introduction, many developing countries are now in the process of founding nuclei of scientific expertise. Close relationship with such groups might further stimulate the objectives mentioned above.

UNIDO and WHO could be instrumental by establishing training courses for the production and control of conventional vaccines in sister institutes in the developed countries. Such courses could widen the existing communication channels and further stimulate the flow of know-how through them.

Finally it should be stressed that the implementation of scientific and economic objectives as described in this report may also be dependant on the structure of the administration, especially in Government Institutes. Modern insights in the structure of an organization indicate that a great deal of independence and freedom to make initiatives should be given to the higher management in the Institute. This can be done by the establishment of a system of contract management wherein control measures are based on the implementation of realistically drafted objectives.

This report deals with the following items which could form the basis for a plan of action based on this model.

(I) Vaccine production technology

(II) Quality control of vaccines including the provision of animal house facilities

(III) Economic aspects of vaccine production and control in the RIVM with some observation on cost-benefit

(IV) Civil engineering of buildings and installations. The lay-out of the units described in this chapter is different from the one actually existing in the RIVM. It reflects a more modern approach then the facilties presently in use, which were constructed about 25 years ago.

(V) A short survey of training programmes on production and control of conventional vaccines by the RIVM in collaboration with international agencies such as WHO and UNIDO

(VI) References

1. VACCINE PRODUCTION TECHNOLOGY

1.1 General concepts

1.1.1 Introduction

In its Expanded Programme on Immunization (EPI) WHO has set as the target complete immunization of all children in the world against the major childhood infectious diseases by the year 1990. Until now only 25 to 30% of the world child population is immunized against these diseases. Local production of the required vaccines will certainly improve the coverage because it will reduce the costs and in particular the requirement for foreign currency to buy the vaccines from outside. In addition it will generate more knowledge on the control and application of vaccines in general.

In this section the general aspects of proven vaccine production technology will be discussed on basis of experiences gained from production of vaccines at the RIVM. In particular attention will be paid to the vaccines against the six target diseases of the EPI of WHO (BCG, diphtheria, tetanus, pertussis, polio, measles) and the tissue culture rabies vaccine. Rabies vaccine has been included because of its general relevance in many developing countries. In view of the low potency and safety of the old brain tissue vaccines replacement by modern tissue culture vaccines would be highly desirable.

1.1.2 Vaccines

Vaccines are prepared for active immunization of man (or animals) against infectious diseases. They should contain or generate the antigen(s) by which a protective immune response against the target disease is elicited. For certain vaccines the required microbial component is well defined such as the toxins of diphtheria and tetanus or polysaccharides of meningococci. In many cases the protective antigen is not exactly known and whole killed bacterial cells (pertussis, cholera, typhoid) or viruses (rabies, polio, influenza) are used as the vaccine while in other cases live attenuated micro-organisms are required for complete protection after immunization (BCG, measles, rubella). Fully chemically defined vaccines are preferable as in general risks of adverse reactions are lower than for whole cell or live vaccines. It is expected that results from biochemical and immunochemical research in combination with the application of modern molecular biology such as recombinant DNA will lead to better defined vaccines. These vaccines will be more specific and cause less adverse reactions upon immunization.

Highly purified vaccines based on extracted antigens or killed organisms have the advantage that they can be applied at optimal concentrations. In this way the number of immunizations can be reduced as recently was demonstrated for diphtheria and tetanus (ref. 7) and inactivated poliovaccine (ref. 8). Further they can be pre-mixed to make a combined vaccine. A good example of this is the DPT-polio vaccine (Diphtheria, Pertussis, Tetanus and polio) as produced at the RIVM which is used in the Dutch National Immunization Programme for more than 20 years. Also for live vaccines combined vaccines exist such as the MMR vaccine which is a mixture of Measles, Mumps and Rubella vaccine. Through the application of more concentrated and combined vaccines the costs of administration of the vaccine, which is at present a high proportion of the total costs of a vaccination programme, should be reduced considerably whilst the immunization coverage is increased.

1.1.3 Required amounts

For the preparation of vaccines the various micro-organisms responsible for the infectious diseases have to be cultivated in order to obtain the basic material. The required primary culture volume per total human dose (THD) depends strongly on the type of vaccine. The culture volumes required for the seven vaccines considered in this report are shown in table 1.1A. It means that for a population of about 10 million and a birth rate of about 3% approximately one million doses of DPT-polio and 400,000 doses of

BCG and measles vaccine are required. In addition it is estimated that for such a population 250,000 doses of plain tetanus vaccine for immunization of people after accidents and pregnant women and approximately 100,000 doses of rabies vaccine for post- and pre-exposure will be required. On basis of these considerations and assuming that an overall recovery of approximately 50% is achieved from crude to final product, the required culture volumes for the different vaccines for a population of about 10 and 100 million have been estimated at the levels given in table 1.1B.

1.1.4 General concept of vaccine production

In comparison with other biotechnological processes the required culture volumes are relatively small. Also in other respects the preparation of the different vaccines has a number of characteristics in common regarding the cultivation processes and processing to a final product. Particularly through the introduction of the homogeneous culture technique using the Bilthoven Unit for the cultivation of micro-organisms, animal cells and viruses, the similarity between the various production processes becomes even more evident. In homogeneous culture the micro-organisms are propagated in stirred fermentors. This culture technique has the advantage that the environmental culture conditions such as temperature, pH and oxygen can be continuously measured and controlled. In this way more consistent production conditions can be obtained and the scaling up of process is facilitated. Moreover, in comparison to stationary cultivation systems in small scale cultivation bottles and flasks the risks of losses by contamination are greatly reduced. Initially the homogeneous culture system was developed and used for the cultivation of bacteria, but through the development of the microcarrier culture system (ref. 9) the cultivation of animal cells and viruses in homogeneous culture systems also became feasible. In microcarrier culture animal cells are grown on small solid particles suspended in the culture medium by stirring. Although this is not a total homogeneous culture system it has all the advantages of the homogeneous culture systems achieved for consistent bacterial growth. Thus, the concept of Vaccine Production as a Unit Process has been introduced at the RIVM by van Hemert (ref. 10), employing similar equipment and techniques for the preparation of different vaccines. As a result, different vaccines can be produced in the same premises with the same equipment and skilled personnel.

In order to comply with the requirements of Good Manufacture Practice (GMP), the production facilities can only be used for production of different vaccines sequentially on the basis of campaigns. Thus, a well documented and validated cleaning and fumigation procedure for the facilities must be established between production runs of the different vaccines. In this way, relatively large scale production units may be used to produce relatively small amounts of vaccines (up to 10 million doses) thus making the production process very economical.

This concept of Unit Processing on a campaign basis sharing the same facilitities has been succesfully introduced for the production of bacterial vaccines (e.g. BCG, diphtheria, pertussis, cholera, typhoid). Tetanus has been excluded because of the WHO requirements that the cultivation and harvesting of tetanus toxin should be performed in a separated unit designated for this purpose because of the hazard from tetanus spores. In our opinion this process could be performed in such a combined production unit for bacterial vaccines provided that strict measures for cleaning and disinfection are employed. Similarly, viral vaccines, for example, inactivated polio and rabies vaccine or the live measles, rubella and mumps vaccine can be produced using the same production facilities. The major advantage of one production unit for different vaccines is not only a reduction in the capital costs of the premises and equipment, but also the larger production batches makes for efficiency in the use of manpower, not only in production but especially in the quality control and routine testing.

1.1.5 Vaccine production facilities

1.1.5.1 General

In scheme 1.1 at the end of this section a flow diagram is shown for each of the vaccines. It reiterates the great similarity between the production processes for the various vaccines. The economic advantages of sharing the production units are clear. Thus, it is only necessary to consider four production units.

1) Production unit for the bacterial vaccines
   (BCG, diphtheria, pertussis and tetanus)
2) Production unit for the inactivated virus vaccines
   (polio and rabies)
3) Production unit for the live virus vaccines
   (measles)
4) Production unit for mixing, filling and packaging

Besides the production units a number of general facilities are required, such as:

1) Administration
2) Central warehouse
3) Civil and technical services
4) Control laboratories
5) Central kitchen

In chapter 4 a lay-out of the required buildings and general technical facilities are described for the production of the 7 vaccines for a population of about 100 million. For production of vaccines for a population of about 10 million, only 25 to 30% less space will be required. The larger capacity can be achieved by using larger equipment such as fermentors, centrifuges etc. which relatively do not take much more space. Thus, the overhead costs will be much higher at a small production capacity.

As the control facilities are further detailed in chapter 2 and the general technical facilities in chapter 4, in this chapter attention will be paid only to the required equipment and personnel of the production units, including the central kitchen. The lay-out of these units is based on experience gained during the last 20 years at the RIVM, but altered in the light of that experience and to comply with GMP regulations. Thus, the production facilities containing living organisms are strictly separated from those involving killed or non-living production by airlocks for personnel and material. Facilities are provided for disinfection of all contaminated materials before leaving the area. These facilities could also be used to sterilise materials being introduced into the areas handling living organisms.

1.1.5.2 Central kitchen

For the cleaning and sterilization of general glassware and the preparation of bulk media and solutions, a general kitchen is planned. The estimated amount of space, budget for equipment and personnel for production of vaccines for respectively a population of 10 and 100 million is given in table 1.1C.

1.1.5.3 Production unit for bacterial vaccines

From the lay-out given in chapter 4 it can be seen that this unit is divided into three parts:

a) The cultivation and harvesting area for the work with live micro-organisms
b) The detoxification, killing and purification area
c) The administrative and general laboratory area

Area a and b can only be entered through special airlocks. All contaminated materials from the cultivation area are disinfected before leaving. The transport of fluids from the cultivation/harvesting to the detoxification/purification area goes through connections in the wall. The estimated amount of space, budget for equipment and personnel required for the production of bacterial vaccines for a population of 10 and 100 million are given in table 1.1D.

1.1.5.4 Production unit for inactivated virus vaccines

This unit can be divided in 4 parts (see chapter 4):

a) The cell cultivation area
b) The cell/virus cultivation and harvesting area
c) The inactivation area

d) The administrative and general laboratory area

Also in this case area a, b and c have separate airlocks for personnel and goods and area b has a disinfection facility. The transport of cell or virus suspensions from one production area to the other goes through connections in the wall. The estimated amount of space, budget for equipment and personnel required for the production of inactivated virus vaccines for a population of 10 and 100 million are given in table 1.1E.

1.1.5.5 Production unit for live virus vaccines (measles)

This unit can be divided into three parts (see chapter 4):

a) The cell cultivation area
b) The virus cultivation and harvesting area
c) Administrative and general laboratory area

In this case area a and b do have separate airlocks for goods and personnel while area b has a disinfection facility. The transport of cells from the cell cultivation area to the virus cultivation area goes through a special airlock. The estimated amount of space, budget for equipment and personnel required for the production of live measles vaccines for a population of 10 and 100 million are given in table 1.1F.

1.1.5.6 Filling and packaging unit

In this unit all vaccines will be mixed, filled and freeze-dried. It has its own facility for washing and sterilizing of ampoules and vials and a restricted area which can only be entered through airlocks for mixing, filling and freeze-drying. For the storage of bulk and packed materials two separated cold rooms are planned. The estimated amount of space, budget for equipment and personnel required for this unit for a population of 10 and 100 million are given in table 1.1G.

1.1.6 Cell substrates for virus multiplication

A critical issue in the preparation of virus vaccines is the cell substrate used for virus propagation. Initially only primary cells - cells which have been cultivated *in vitro* only once - were accepted for this purpose. At the end of the sixties also human diploid cells (HDC) - which are cells originating from embryonic human tissue and have a limited life span of about 50 generations and maintain a normal diploid character throughout its life span - were accepted. Recently also some non-tumorigenic, well characterised continuous cell lines have been accepted in some countries for the production of highly purified inactivated virus vaccines provided that the cellular DNA in the final product is reduced by an adequate purification process to picogram (pg) levels. Although WHO has formulated the minimum requirements for the production of inactivated polio vaccine on such cell substrates (ref. 11) an exact minimum level in pg per dose is not indicated. However, a level of 10 to 100 pg per dose of vaccine is generally considered to be acceptable. Meanwhile inactivated polio vaccine produced on this cell substrate has been accepted in France while in a number of countries the application for registration has been submitted.

Although at the RIVM the inactivated polio and rabies vaccine are presently produced on respectively tertiary monkey and dog kidney cells this report will anticipate this new development. Therefore, the production of polio and rabies vaccine will be described using a particular (non-tumorigenic) continuous cell line, VERO cells.

For the production of measles vaccines chick embryo fibroblast cells were chosen, cells which grow in standard monolayer culture systems, although in some countries also human diploid cells are used.

The key advantage of VERO and HDCS (MRC 5) cells is that they can be maintained as a well characterised Master Cell Bank. This removes the necessity to house and maintain in isolation animals for primary cell cultivation.

1.1.7 Preparation of final vaccine

In contrast to many other countries where the combined DPT-vaccine and live poliovaccine are used for the National Immunization Programmes, in The Netherlands the combined DPT-polio vaccine is used as inactivated polio can be combined with the DPT-vaccine. In this report the Dutch approach will be followed which means that the DPT-polio vaccine will be described. The composition of this vaccine is given in table 1.1H. Besides this also the preparation of plain polio, adsorbed tetanus and freeze-dried rabies and measles vaccine will be described.

1.1.8 Equipment

In table 1.1.K a list of the main equipment used at RIVM for the production of bacterial and virus vaccines is given. The fermentation equipment was originally developed for the production of bacterial vaccines by van Hemert (ref. 10) at the RIVM in the beginning of the sixties. Together with ancillary equipment, it is now produced by the Dutch Company, Contact-Flow B.V., under the name "Bilthoven Unit System." It has the advantage of being a fully standardised system which has been proved for the cultivation of bacteria, cells and viruses. Many other companies also market equipment for biotechnological processes including fermentors, control equipment, autoclaves, freezers, centrifuges and other items in 1.1.K. A more extensive survey of the different companies from which this type of equipment may be purchased can be found in reviews given by WHO (ref. 12).

Table 1.1A

Required amount of culture volume for one total human dose (THD)

Vaccine	Type	Number of doses	Culture volume (ml)
BCG	live bacteria	1	0.01
Measles	live virus	1	1.0
Diphtheria	inactivated toxin	3	0.4
Tetanus	inactivated toxin	3	1.0
Pertussis	inactivated whole cells	3	1.5
Polio 1	inactivated virus	3	3.0
2	inactivated virus	3	1.5
3	inactivated virus	3	3.0
Rabies pre-exposure	inactivated virus	3	30
post-exposure	inactivated virus	6	60

Table 1.1B

Required culture volumes for production of vaccines for a population of respectively 10 and 100 million

Vaccine type	Antigen content per dose	Crude antigen yield/ml	Required culture volume at a population of 10 million	100 million
D	15 Lf	200 Lf	150 l	1500 l
P	16 IOU	60 IOU	500 l	5000 l
T	10 Lf	50 Lf	500 l	5000 l
BCG			7 l	70 l
Polio 1	40 DU	80 DU	1000 l	10000 l
2	8 DU	30 DU	750 l	7500 l
3	32 DU	60 DU	1000 l	10000 l
Measles	$10^{4.0}$ PFU	$10^{4.5}$ PFU	400 l	4000 l
Rabies	2.5 IU	0.5 IU	1000 l	10000 l

Lf : flocculation units

IOU : International Opacity Units

DU : D-antigen Units

IU : International Units

PFU : Plaque Forming Units

Table 1.1C

Required amount of space, budget for equipment and personnel for the Central Kitchen

		Population of	
		10 million	100 million
Space		360 m²	520 m²
Budget for equipment in Dutch guilders		1.5×10^6	2.0×10^6
Personnel:	chemist	1	1
	senior technicians	2	3
	technicians	2	5
	other personnel	6	10

Table 1.1D

Required amount of space, budget for equipment and personnel for the production unit of bacterial vaccines

		Population of	
		10 million	100 million
Space		360 m²	520 m²
Budget for equipment in Dutch guilders		1.5×10^6	2×10^6
Personnel:	biotechnologist	1	1
	chemist	1	1
	senior technicians	2	3
	technicians	3	4
	other personnel	3	4

Table 1.1E

Required amount of space, budget for equipment and personnel for the production unit of inactivated virus vaccines

		Population of	
		10 million	100 million
Space		415 m²	620 m²
Budget for equipment in Dutch guilders		2×10^6	2.75×10^6
Personnel:	biotechnologist	1	1
	chemist	1	1
	senior technicians	2	3
	technicians	3	5
	other personnel	3	5

Table 1.1F

Required amount of space, budget for equipment and personnel for the production unit of live virus vaccines

		Population of	
		10 million	100 million
Space		360 m²	470 m²
Budget for equipment in Dutch guilders		1×10^6	1.5×10^6
Personnel:	virologist	1	1
	chemist	1	1
	senior technicians	2	3
	technicians	2	4
	other personnel	2	4

Table 1.1G

Required amount of space, budget for equipment and personnel for the filling and packaging unit

	Population of	
	10 million	100 million
Space	625 m²	930 m²
Budget for equipment in Dutch guilders	3×10^6	4×10^6
Personnel: chemists	2	2
senior technicians	2	3
technicians	2	4
other personnel	6	10

Table 1.1H

Composition of DPT-polio vaccine per dose

Diphtheria	15 Lf
Pertussis	16 IOU
Tetanus	10 Lf
Polio type 1	40 DU
type 2	8 DU
type 3	32 DU
$AlPO_4$	2 mg
2-phenoxyethanol	5 mg
formaldehyde	25 µg
sodium chloride	isotonic

Table 1.1K

Vaccine production equipment used at RIVM

1. Cultivation

Medium vessels	Contact-Flow, Ridderkerk, Netherlands
Control Unit for temperature and pH control of medium vessels	Contact-Flow, Ridderkerk, Netherlands
Freezer -70°C	
Forma Bio Freezer	Forma Scientific Mariette Ohio, USA
LAF-cabinet, Biohazard	Clean Air Techniek, Woerden, Netherlands
Incubator	Kötterman Hetze Hänigsu, Germany
Rotary-shaker	New Brunswick Sc., New Brunswick, USA B.Braun, Melsungen, Germany
Freeze-dryer, small	Edwards Vacuum Ltd, Crawley, England
Bioreactors 3, 10, 50, 200 and 500 l	Contact-Flow, Ridderkerk, Netherlands
Sterigas 100 for exhaust gas disinfection	Contact-Flow, Ridderkerk, Netherlands
Control Unit for control of bioreactors	Contact-Flow, Ridderkerk, Netherlands
Pump for emptying vessels:	
Lutz type B3	Lutz, Wertheim, Germany
High Pressure Cleaning Device	Kärcher, Winnenden, Germany
Trypsinization apparatus for cell culture	Contact-Flow, Ridderkerk, Netherlands
Sanitary T-pieces	Contact-Flow, Ridderkerk, Netherlands
Unit process vessels, 100 l, stainless steel storage vessels	Contact-Flow, Ridderkerk, Netherlands
Vibromixer E2	Chemap, Männedorf, Switzerland
Vibromixer support	Contact-Flow, Ridderkerk, Netherlands
B20, B10 containers: 20 l, 10 l bottles with standardized stainless steel flanges	Contact-Flow, Ridderkerk, Netherlands

Infuse bottles of 3, 1, 0.5 and 0.25 l content	Schott, Mainz, Germany
System caps for aseptic operation with 3, 1, 0.5 and 0.25 l bottles	Contact-Flow, Ridderkerk, Netherlands
Breathing filters Acrodisc	Gelman Science, Ann Arbor, Michigan, USA
Peristaltic pumps and silicon tubing	Watson and Marlow, Falmouth, England

2. Harvesting

Seitz-filter	Seitz GmbH, Bad Kreuznach, FRG
Meta-filter (diphtheria, polio)	Contact-Flow, Ridderkerk, Netherlands
Sterile filter (0.2 μ) Pall	Pall Corp., New York, USA
Hyflo filteraid	John Manville, Denver, USA
Continuous aseptic centrifuge	Westfalia, Oelde, Germany
Batch centrifuge, Cryofuge 6-6	Heraeus Christ, Osterode, Germany
Ultrafiltration cartridges	Amicon Corp., Danvers, Mass.,USA
Neo Ultron Ultrafiltration system	Contact-Flow, Ridderkerk, Netherlands
Dispensing apparatus (Pertussis, BCG)	Contact-Flow, Ridderkerk, Netherlands
Clamb-blox (or semi-automatic operation of dispensing apparatus)	Contact-Flow, Ridderkerk, Netherlands
Shaker for resuspension	Heidolph, DSG302, Kelheim, Germany
pH-meter, Philips PWE9409/21	Philips, Eindhoven, Netherlands
Protein resistant pH probe	Russell, Auchtermachty, Scotland
Extinction meter for O.D.measurement Vitatron type UPS	Vitatron, Dieren, Netherlands
Ramon-bath for flocculation of diphtheria and tetanus toxoid	Contact-Flow, Ridderkerk, Netherlands
Balance: Metler PC4400	Metler, Zürich, Switzerland

3. Mixing and Filling

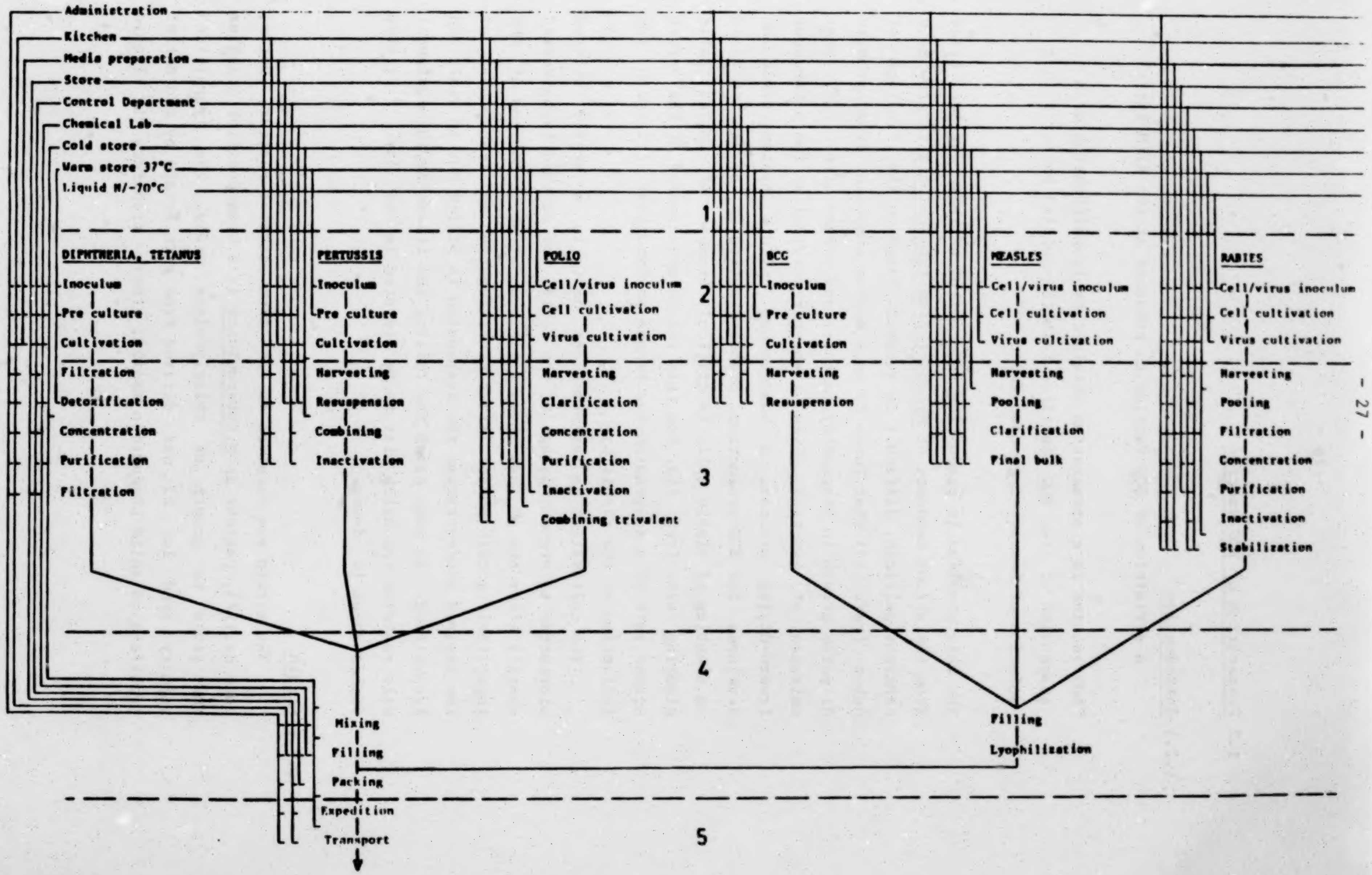
Mixing jacketed vessel and Control Unit	Contact-Flow, Ridderkerk, Netherlands
Strunck filling apparatus	Strunck, Köln, Germany
Bausch und Stroebel filling, washing, labelling machine	Bausch und Stroebel, Ilshofen, Germany

4. General Services

Steam generator, Clayton type EG33	Clayton, Bornem, Belgium
Autoclaves	Linden, Zwijndrecht, Netherlands
Air compressor oil free KCTD 200-250	Kaeser, Coburg, Germany
Cool dryer	VIA, Giethart, Hengelo, Netherlands
Vacuum Unit	Leybold Hereaus, Köln, Germany
Gasstation for O2, N2, CO2 gas	Hoekloos, Amsterdam, Netherlands
Boilers, Sentry SWF-75	Homas, Nijkerk, Netherlands
Demi-water	Elga group, Bucks, England
Aquadest installation	Aqua Finn, Helsinki, Finland
Hot air sterilizers	Hereaus, Hanau, Germany

Scheme 1.1

FLOWDIAGRAM OF THE PRODUCTION PROCESSES.



1.2 Preparation of BCG vaccine

1.2.1 Introduction

A definition of BCG vaccine as produced at the RIVM is:

> "BCG vaccine is a preparation obtained by lyophilization of a suspension of live BCG (Bacillus Calmette-Guérin) bacteria in a solution of Haemaccel-glucose-Tween 80"

The main problems in production and control of this vaccine arise from the strong tendency of Mycobacterium tuberculosis to grow as a coherent pellicle, difficult to suspend. Based on the findings of Dubos (ref. 13) that Tween 80 as a medium component could promote disperse growth in stationary cultures and Ungar (ref. 14) that omission of glycerol gives a better survival at the subsequent freeze-drying process, a homogeneous culture system could be developed for BCG-production. Standardization of the vaccine based on counting of viable cells is difficult because of unpredictable clumping size (ref. 15). For this reason measurement of the oxygen uptake rate of a suspension has become an important criterion for indication of the viability.

The cultivation is performed at 36 °C in a gently stirred bioreactor to prevent damage of the cells. The culture is harvested shortly after the pO_2 has shown a minimum, at about the end of the logarithmic growth phase. After centrifugation and resuspension at the desired concentration the suspension is filled out in vials and lyophilized. In many cases the filling and freeze-drying equipment will restrict the batch size to be operated in one day: 7 litres yields about 10^6 doses.

1.2.2 Strain

The strain was received in 1964 from Institut Pasteur, Paris, code nr.1173, Pasteur 2; M.tuberculosis is a Gram-positive organism that gives no growth on thioglycolate media. The lyophilized primary seed lot P3 was derived from this strain by two serial transfers on solid Löwenstein medium, three transfers in liquid

Dubos medium and one transfer in liquid Ungar medium. It is stored at -70°C. From this primary seedlot a homogeneous 40 l culture in Ungar medium was made, filled out in 150 ml portions in 500 ml bottles, frozen in ethanol/CO_2 and stored at -70°C. These bottles are used as a working seed lot.

1.2.3 Cultivation

1.2.3.1 Media

1. Production medium (Ungar's medium)

   One litre of the modified Ungar medium to be dissolved in distilled water in the given order contains the following ingredients:

KH2PO4	1	g
Na2HPO4.2H2O	2.5	g
Ferriammonium citrate	0.1	g
Monosodium glutamate	4	g
L-Asparagine	4	g
L-Glutamine	4	g
Casitone	1	g
Tween-80	0.5	g
CaCl2.2H2O	1.32	mg
CuSO4.5H2O	0.78	mg
ZnSO4.7H2O	0.89	mg
MgSO4.7H2O	10	mg

   Sterilize at 110°C for 20 minutes.

2. Resuspension medium

   One litre of this medium, prepared with distilled water and sterilized at 120°C for 20 minutes, contains:

glucose	50	g
Tween 80	0.05	g
Haemaccel	83	g

1.2.3.2 Culture

The modified Ungar medium is sterilized in the bioreactor at

110 °C for 20 min. For inoculation of a 7 l culture one bottle from the primary seed lot is directly used after thawing in a waterbatch of 35 °C.

The cultivation temperature is 36 °C. The stirrer speed and air flow rate are increased gradually on guidance of the turbidity from respectively 50 to 200 rpm and 1 to 3 l per min. A typical graphic representation of a BCG cultivation is given in figure 1.2.

1.2.4 Processing

The culture is harvested within 24 hours after the pO_2- level has reached its lowest level, by dispensing the culture-fluid aseptically in centrifuge bottles. Centrifugation is performed in cooled centrifuges at 2400 g for 60 minutes. The sediment is resuspended to a suitable concentration, normally to 6x the original cultivation volume, depending on viable count and oxygen uptake rate in resuspension medium. This suspension is dispensed aseptically into sterile ampoules of 0.5 ml or vials of 5 ml and lyophilized.

FLOW SHEET OF THE PREPARATION OF FREEZE-DRIED BCG-VACCINE (1.2)

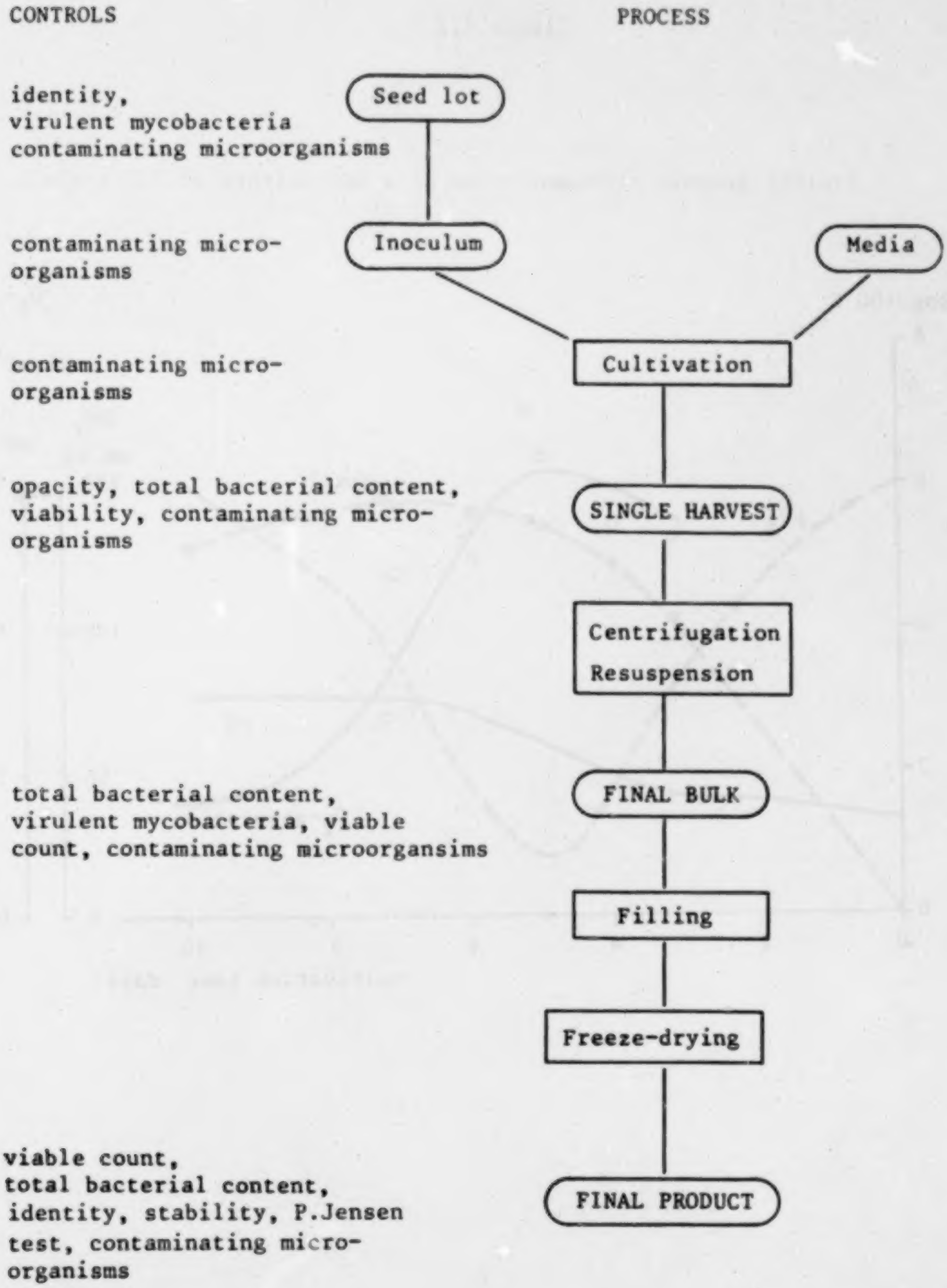
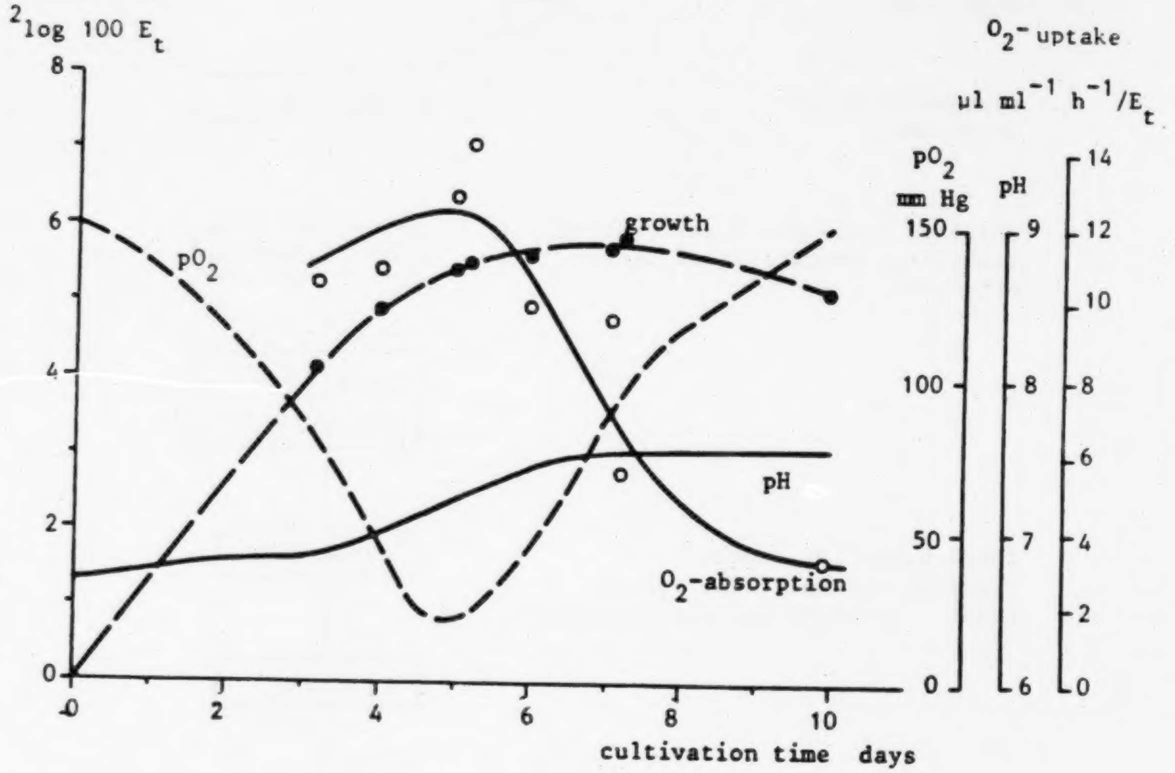

CONTROLS
PROCESS
identity, virulent mycobacteria contaminating microorganisms
Seed lot
contaminating micro-organisms
Inoculum
Media
contaminating micro-organisms
Cultivation
opacity, total bacterial content, viability, contaminating micro-organisms
SINGLE HARVEST
Centrifugation
Resuspension
total bacterial content, virulent mycobacteria, viable count, contaminating microorgansims
FINAL BULK
Filling
Freeze-drying
viable count, total bacterial content, identity, stability, P.Jensen test, contaminating micro-organisms
FINAL PRODUCT

Figure 1.2

Typical graphic representation of a BCG culture at 7 l scale



1.3 Preparation of pertussis vaccine

1.3.1 Introduction

A definition of Pertussis vaccine as produced at the RIVM is:

> "Pertussis Vaccine is a suspension of killed micro-organisms of Bordetella pertussis, strain 509 (agglutinogen 1, 2, 6 and 7) and strain 134 (agglutinogen 1, 3 and 7), in physiological saline with hyamine at a concentration of no more than 1:60,000"

Development of a satisfying large scale process for production of pertussis vaccine has been hampered by several factors:

1. lack of basic knowledge about the identity of the protective antigen(s)
2. inaccurate test for determination of protective values of a given preparation
3. lack of a test model predicting side-reactions after immunization

Recently the work of Sato et al. has resulted in the development of an acellular pertussis vaccine that has, however, not yet been used for mass vaccination outside of Japan (ref. 16). Moreover, no controlled trial of safety and efficacy has yet been reported.

The homogeneous cultivation method is used at RIVM (ref. 17). The medium used is a modified Cohen and Wheeler-medium (ref. 18) on which for both strains cell concentrations up to 60 x 10^9 cells/ml are obtained with a mouse protecting activity of 5 to 12 IU per human dose of 16 IOU.

Harvesting is done by centrifugation since comparative tests with acid-precipitation indicated a lower mouse protecting activity and a lower weight gain in the mouse toxicity test (ref. 19, 20), using an aseptic continuous centrifuge. Detoxification is carried out by a heat-treatment for 10 minutes at 56 ^{o}C.

1.3.2 B.pertussis strains

Two strains are used for the routine preparation of pertussis vaccine:

strain 509 (containing the agglutinogens 1, 2, 6 and 7) from the

culture collection of RIVM
strain 134 (containing the agglutinogens 1, 3 and 7) originally obtained from Dr.Pillemer (Lederle).

The strains are kept in the lyophilized state at room temperature. Both strains grown on Bordet-Gengou solid medium containing sheep blood at 35 °C, show smooth glistening colonies of about 1 mm in diameter after 3 x 24 hours. The Gram-smear shows Gram-negative coccoid rods. No growth should be observed on nutrient broth.

For lyophilization to produce a seed suspension of bacterial cells they are harvested from a regular culture in the logarithmic phase and re-suspended in sterile milk to a concentration of 250-500 x 10^9 cells per ml.

1.3.3 Cultivation

1.3.3.1 Media

1. Preculture medium

One liter of this so-called Verwey-medium contains the following components:

Casamino acids (Difco, technical grade)	14	g
KCl	0.200	g
KH_2PO_4	0.500	g
$MgCl_2.6H_2O$	0.100	g
Nicotinic acid	0.020	g
Glutathione	0.010	g
Starch	1	g
Distilled water		

Prepare a starch suspension by stirring the starch in 500 ml distilled water while heating. Add the starch to the rest of the quantity of distilled water, in which the other components already have been dissolved. Adjust the pH to 6.8 - 6.9.

The medium is dispensed in 200 ml quantities in 500 ml bottles for inoculation of 40 l production cultures, in 400 ml quantities in 1 l bottles for 140 l production cultures and in 800 ml quantities in 3 l bottles for 350 l production cultures. The bottles are closed with cotton wool plugs and sterilized at 120 °C for 20 minutes.

2. Production medium (modified Cohen and Wheeler medium)

One liter of this so-called B-2 medium contains the following ingredients:

Casamino acids (Difco, technical)	6	g
Starch	1.5	g
L-Glutamic acid	5	g
NaCl	2.5	g
KH_2PO_4	0.5	g
$MgSO_4.7H_2O$	0.1	g
$CaCl_2.6H_2O$	0.01	g
$FeSO_4.7H_2O$	0.0125	g
$CuSO_4$	0.0005	g
Glutathione	0.01	g
Yeast extract (Gistex, Gist-Brocades)	50	ml
Distilled water		

The medium components are prepared in concentrated form in three solutions and can be stored at 4 °C:

a) 3% starch solution, sterilized at 110 °C for 30 minutes

b) 10% casamino acids solutions, filtered at pH 7.2 through filter paper and sterilized at 120 °C for 20 minutes

c) main stock solution: a sterile filtered 6.67 times concentrated solution containing the rest of the medium components, including one third of the casamino acids.

For preparation of the final medium these three solutions are aseptically combined, diluted with sterile distilled water in the bioreactor and heated at 110 °C for 20 minutes.

1.3.3.2 Inoculum

A lyophilized culture is seeded in 200 ml Verwey-medium in bottles of 500 ml and incubated on a rotary shaker at 35 °C for 3 to 4 days. The culture is checked for growth and purity, transferred into Verwey medium at a dilution rate of about 1 to 100 and placed on a rotary shaker at 35 °C for 24 hours. After growth and purity have been checked, the cultures are used for the inoculation of the production cultures.

1.3.3.3 Culture

The medium is filled in a sterilized bioreactor and given a heat treatment at 110°C for 20 minutes. After inoculation with seed culture the air flow rate is adjusted at 5 l per min and the stirrer speed at 750 rpm for a 40 l culture, and at 10 l per min and 300-400 rpm for a 140 l culture. At a pH of 8.2 (40 l) or 8.0 (140 l) the culture is automatically cooled down to about 10°C, awaiting the harvesting. A typical graphical representation of the production is given in figure 1.3.

1.3.4 Harvesting (batch)

After aseptic filling in 500 ml bottles, the culture is centrifuged in a cooled centrifuge at about 2400 g for 60 minutes. The supernatant is aseptically removed: since the supernatant could contain a thermostabile toxin this step has to be carried out carefully. The cells are resuspended in 0.85% NaCl solution with hyamine 1:60,000 for use in DPT-polio vaccine or merthiolate 1:10,000 for use in DPT-vaccine. These suspensions are collected in 10 or 20 l bottles and stored at 4°C. Samples for sterility and protective activity testing are taken. If the results of the test are favourable, the lot is mixed with a similar lot of the other pertussis strain to form a mixed batch of both strains. This suspension is diluted with 0.85% NaCl plus preservative to a concentration of 200 IOU per ml and heat inactivated at 56°C for 10 minutes. After cooling samples are taken for quality control. The suspension is transferred from the heating vessel into 20 l containers and stored at 4°C. After one week storage at 4°C samples are taken from each bottle for sterility control.

FLOW SHEET OF PERTUSSIS VACCINE PRODUCTION (1.3)

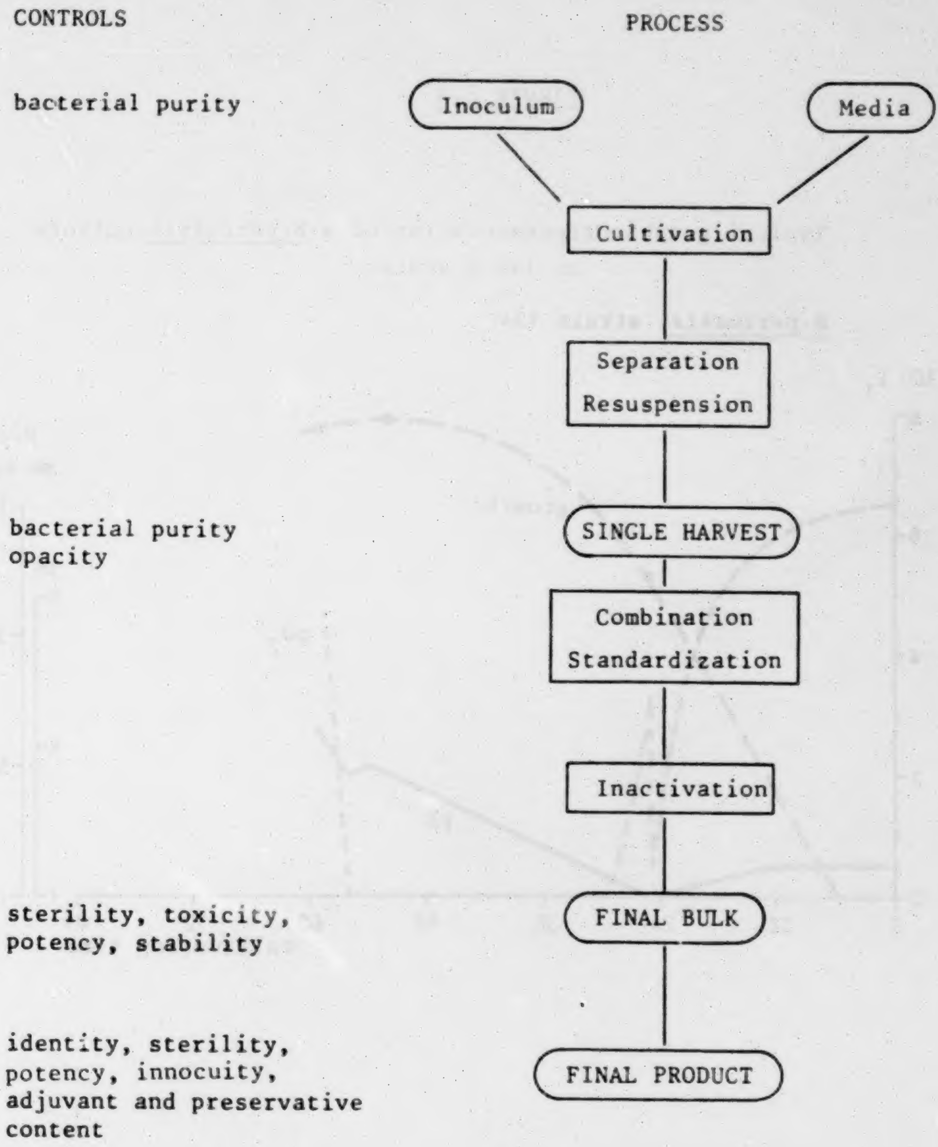
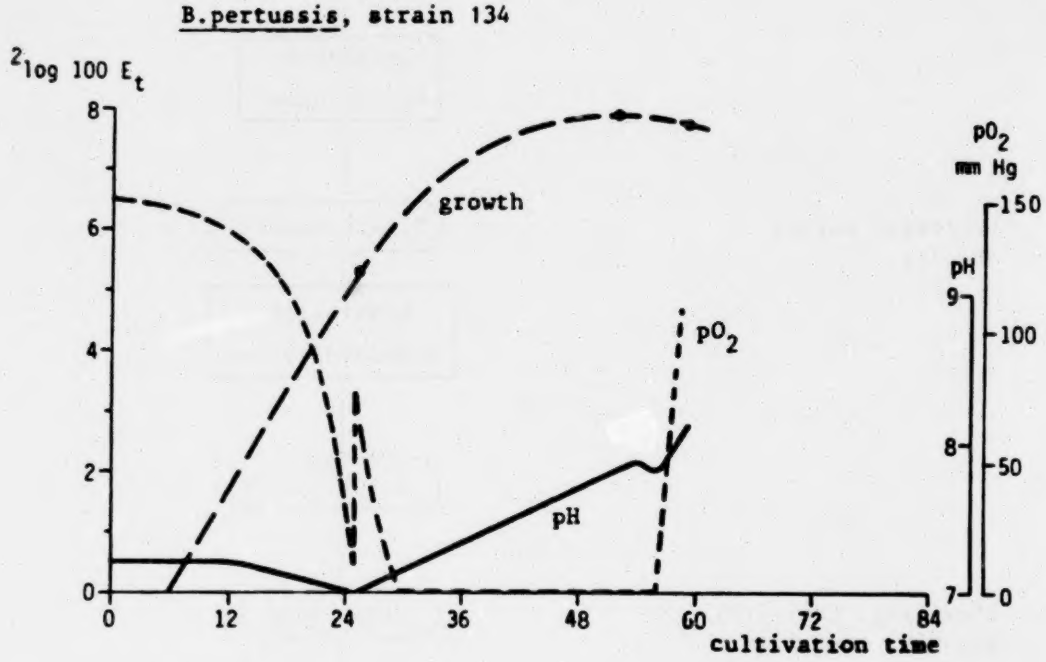


Figure 1.3

Typical graphic representation of a B.pertussis culture at 140 l scale



1.4 Preparation of purified diphtheria toxoid

1.4.1 Introduction

A definition of purified Diphtheria Toxoid as produced at the RIVM is:

> "Purified diphtheria toxoid is a concentrated sterile solution of formalin detoxified and ammonium sulphate purified toxin of Corynebacterium diphtheriae in a physiological saline solution"

Diphtheria toxin is prepared in homogeneous culture in the Bilthoven Unit System at production scales of 40 l, 150 l and 350 l culture volume. Two types of media can be used. The usual medium based on Linggood contains papain digested meat (ref. 21); alternatively, a better defined semi-synthetic medium, with NZ-Amine as the main protein source (ref. 22) can be used. In this report only the production on Liggood medium will be described as all technical problems met with the use of Stainer medium have not been solved yet. Toxin synthesis appears to be inhibited by iron concentrations over 1000 mg/l in the medium (ref. 23) and dissolved oxygen concentration below 10% of air saturation during cultivation (ref. 10). To prevent excessive foaming a vortex type of mixing is used in combination with gass flushed over the culture avoiding any sparging. After a total cultivation time of 40 to 48 hours an average toxin titer of 200 Lf/ml is obtained. The culture is then clarified, either by filtration after addition of diatomaceous earth as filter-aid or by separation in a continuous separator. After sterile filtration over 0.2 u membrane filters the filtrate is detoxified by addition of 0.6% formalin and by incubation at 35 °C for 6 weeks. Several batches of diphtheria toxoid are combined for ultrafiltration, ammonium sulphate fractionation and sterile filtration.

1.4.2 Strain

The strain of C.diphtheriae used is the Park Williams strain no. 8. It was obtained from the Wellcome Research Laboratories, bearing the code CN 2000. C.diphtheriae is strongly pleomorphic. The Gram-smear mainly shows Gram-positive, slightly bent rods lying together in groups, giving the impression of Chinese characters. The strain ferments glucose rapidly and maltose relatively slow. Lyophilization is carried out with a suspension in 50 ml skimmed milk of colonies scraped from an Loeffler plate which has been incubated at 35°C for 24 hours.

1.4.3 Cultivation

1.4.3.1 Media

Preparation of 100 litre Linggood medium:

Ingredients:

Beef meat (without fat)	15.5	kg
NaOH-solution 50%	c. 800	ml
Papain	7 x 22	g*
Cysteine.HCl	31.5	g
HCl 35%	c. 250	ml
Glacial acetic acid	c. 600	ml
Yeast extract (Gistex, Gist-Brocades)	15	g
Sodium lactate solution 60% w/v	150	ml
Maltose	2500	g
MgSO4.7H2O	28.3	g

Growth factors according to Mueller:
62 g MgSO4.7H2O, 175 mg β-alanine, 175 mg nicotinic acid, 11 mg pimelic acid, 75 mg CuSO4.5H2O, 60 mg ZnSO4.7H2O, 22 mg MnCl2.4H2O

*The extact amount of papain is dependent upon the batch used. Before a new batch of papain is taken into use, a series of cultivation experiments are made to determine the optimal amount to be used for the digestion of the meat.

Preparation procedure:

Remove all fat from about 18 kg fresh meat, and mince the meat through a meat mincer with 3 mm holes. Take 15.5 kg of the minced meat and mix with 85 litres tap water by stirring 5 to 10 minutes. Take away the fat particles using filter paper or a fine sieve. Adjust the pH to 7.0 with 50% NaOH solution. Add 31.5 g cysteine hydrochloride, dissolved in 150 ml distilled water (giving a 0.002 M solution in 100 litres), and warm up under stirring to 50°C. Remove the fat collected at the rim of the vessel with filter paper. Adjust the pH again to 7.0 if necessary. All pH measurements should be performed at 25°C.

Digest the meat while stirring during 7 x 0.5 hour adding 22 g papain every half hour. Keep the pH at 7.0-7.1 during the digestion, and the temperature during the first five phases at 50°C. Bring then the temperature to 55°C during the 6th phase, and to 60°C during the 7th phase.

Hereafter the pH is adjusted to 5.1 with concentrated HCl. Then boil during 10 minutes. Cool to 60°C, and adjust the pH to 4.1 with glacial acetic acid. Filter the broth through course filters, and keep the filtrate overnight at 4°C. Adjust the pH to 7.6 with 50% NaOH-solution.

Add the following components:

Yeast extract (Gistex, Gist-Brocades)	15 g
Sodium lactate solution 60% w/v	150 ml
Growth factors according to Mueller	150 ml
Maltose	2500 g

Adjust now the volume with tap water to 100 l, and adjust the pH with 50% NaOH solution to 7.8. Add: 28.3 g $\mathrm{MgSO4.7H2O}$, dissolved in 100 ml water.

Filter the solution through 5 Seitz K5 and 9 EKS filter sheets. Discard the first 10 litres and collect the rest aseptically in sterile containers. Take a sample for sterility control (200 ml) at the end of the filtration.

1.4.3.2 Inoculum

The content of a lyophilized seed culture is transferred to a Loeffler serum plate, and incubated at 35°C for 48 hours. From this plate colonies are transferred to 100 ml Linggod medium in 500 ml bottles and shaken on a rotary shaker at 35°C for 24 hours.

After checking the purity of the inoculum on a Gram-smear, the production cultures are inoculated at a volume ratio of about 1:400.

1.4.3.3 Culture

After filling of the sterile medium aseptically into the sterilized bioreactor, about 0.01% sterile antifoam solution (silicone MS, antifoam A, 25% w/v in liquid paraffin) is added. After heat treatment for 20 minutes at 100 °C a sample is taken from the bioreactor for determination of extinction and pH. After correction of the pH-controller the pH of the medium is brought to 7.8 with concentrated NH_4OH. For 40, 150 and 350 l culture volumes the air flow rates and stirrer speeds are respectively: 5, 10, 10 l/min and 1000, 500, 300 rpm. Oxygen is supplied to the gas phase if the dissolved oxygen concentration (D.O.) of the culture is below 10% air saturation. Excessive pH-increase, i.e. 0.1 pH-unit over the 18 hours cultivation value, is controlled by addition of a 10% glucose solution. Harvesting of the culture is made after 40 to 48 hours when the Lf value is no longer increasing and has reached a value of about 200 Lf/ml. A typical representation of the growth of a production culture is given in figure 1.4A. On harvesting the culture is checked for purity be means of a Gram-smear.

1.4.4 Processing

1.4.4.1 Clarification

Two methods for separation of cell mass and culture fluid may be used depending on the volume to be handled and the equipment available: filtration or continuous centrifugation. For effective filtration the culture fluid is mixed with 4% filter-aid (diatomaceous earth) and filtered over a Meta-filter which is a course mesh of preferentially stainless steel candles at the surface of which the filter-and can build up. The clear filtrate and the physiological saline used for washing the filter after filtration, is collected and filtered through a prefilter and then a sterile filter of 0.22 μm pore size. Samples are taken for sterility and determination of toxin concentration. For clarification of large culture volumes (over 200 l) application of a continuous centrifuge with a capacity of about 200 l/hr is advantageous. After centrifugation the fluid is filtered through a prefilter and then through a sterile filter of 0.22 μm pore size.

1.4.4.2 Detoxification

The crude toxin is detoxified by addition of 2x 0.3% v.v. of a 40% w/v formaldehyde solution, directly and at the third day after harvesting and incubated for six weeks at 35 °C. The pH of the fluid at the first resp. third day should be about 7.3 resp. 7.0. After this period the container is placed in the refrigerator at 4 °C awaiting the release after completion of the quality control tests.

1.4.4.3 Purification

The purification of diphtheria toxoid comprises:

a. concentration of the crude toxoid by ultrafiltration through hollow fiber filter cartridges
b. fractionation with ammonium sulphate followed by dialysis
c. sterile filtration

The detailed procedures are given below.

Ultrafiltration with hollow fiber filters

The basic idea of this type of cross-flow filtration is that the culture fluid is circulated under pressure through permeable hollow fibers with a pore size (cut-off) below the molecular weight of the component which should be concentrated. Although the molecular weights of diphtheria and tetanus toxoid are about 70.000 and 150.000 respectively the cut-off of the ultrafilters should be about 10.000 to prevent excessive losses. A schematic presentation of a ultrafiltration apparatus is given in figure 1.4B.

Before operation the whole equipment has to be sterilized by heat (steam at 120 °C). After rinsing with sterile distilled water the apparatus is connected to an in- and outlet of the container with toxoid. The maximum operating pressure on the hollow fiber should not exceed 25 p.s.i., and the mean transmembrane pressure (m.t.p. 1/2 x [P-inlet + P-outlet]), should not exceed 20 p.s.i. With a m.t.p. of 10 p.s.i. an ultrafiltration rate of 10-15 l per hour per cartridge can be obtained. The ultrafiltrate leaving the system via the manifold is controlled for absence of toxoid.

After achieving the desired degree of concentration (10 to 15 times) the concentrated toxoid is pumped in a suitable container and the entire system rinsed with a sterile physiological saline solution. This washing fluid is collected in the same container.

Purification of the concentrated toxoid

The crude toxoid is purified by means of ammonium sulphate precipitation. First the toxoid has to be diluted to 1500 - 2000 Lf/ml to improve the purity of the endproduct. To determine the ammonium sulphate concentrations at which the bulk of the toxoid will precipitate a pilot study has to be done with small quantities of toxoid.

To samples of 25 ml of toxoid increasing quantities of ammonium sulphate are added. For diphtheria toxoid these quantities are 5.50, 6.25, 7.00, 7.75, 8.50 and 9.25 g. For tetanus toxoid 2.25, 2.75, 3.50, 4.25, 5.00 and 5.75 g. After one hour the suspensions are centrifuged and the sediments are dissolved to 25 ml with saline. The toxoid concentrations in these solutions are determined by Ramon-flocculation. The results are plotted against the corresponding ammonium sulphate concentrations. From the curve obtained in this way the ammonium sulphate concentrations at which the bulk of the toxoid precipitates, are determined.

To the crude toxoid the calculated amount of ammonium sulphate, corresponding to the lower concentration determined in the pilot precipitation study, are added. After dissolution of the salt by shaking, the toxoid is placed at room temperature. The next day, the precipitate will have sedimented partially. The clear supernatant is siphoned off as much as possible, and the rest of the suspension is clarified by centrifugation. The sediment is discarded. To the clear supernatant fluid a second quantity of ammonium sulphate is added, so that the higher concentration determined in the pilot study is reached. After the salt has dissolved, the suspension is placed at room temperature to precipitate the sediment. The next day, the supernatant fluid is siphoned off. The precipitate is collected by centrifugation and is dissolved in saline. The solution is clarified by filtration and residual ammonium sulphate is removed by gelfiltration on Sephadex G50. Finally the purified toxoid is filtered through sterile filters of 0.22 µm pore size.

FLOW SHEET DIPHTHERIA TOXOID PRODUCTION (1.4)

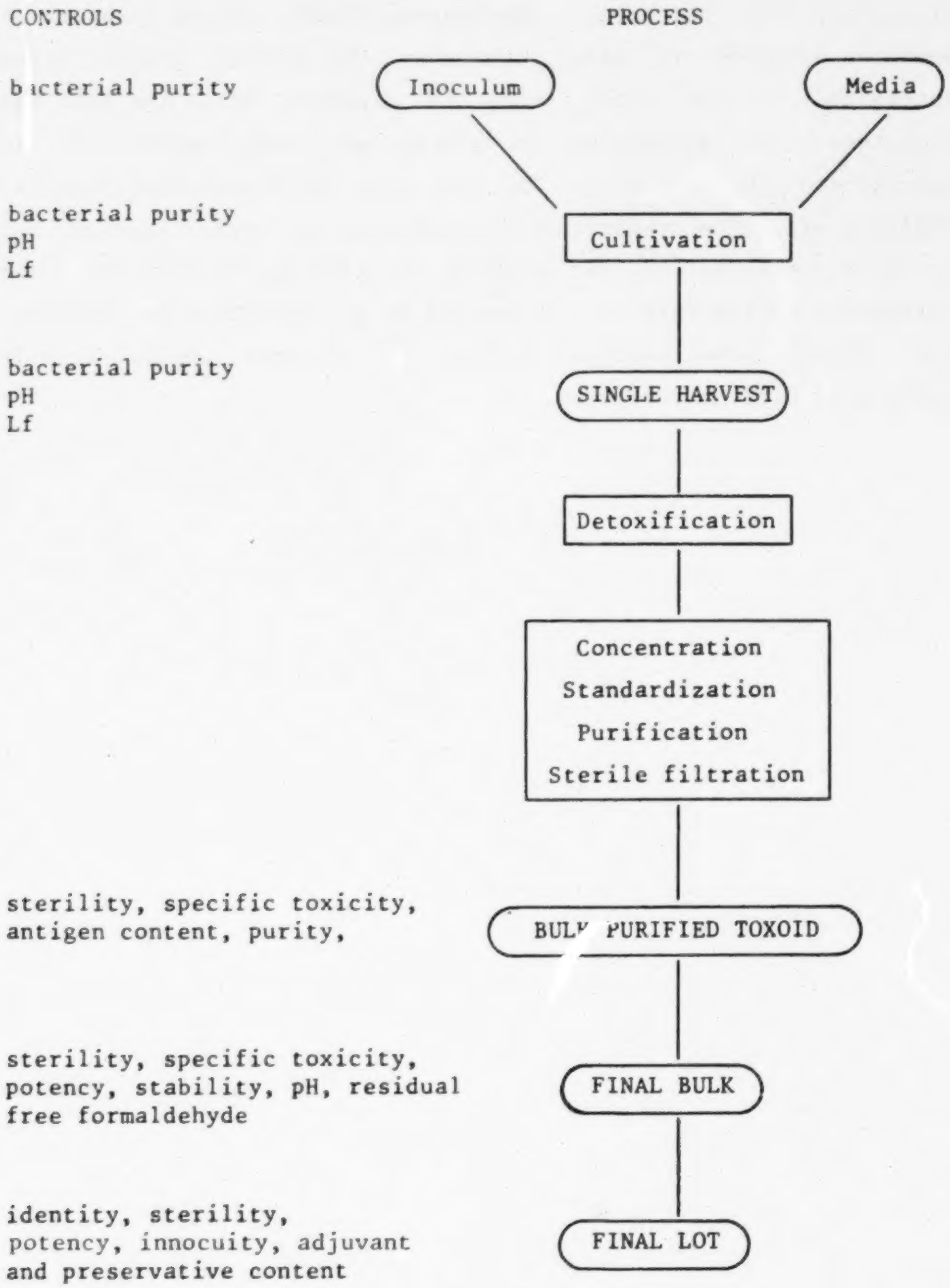


Figure 1.4 A

Typical graphic representation of a C.diphtheriae culture at 140 l scale

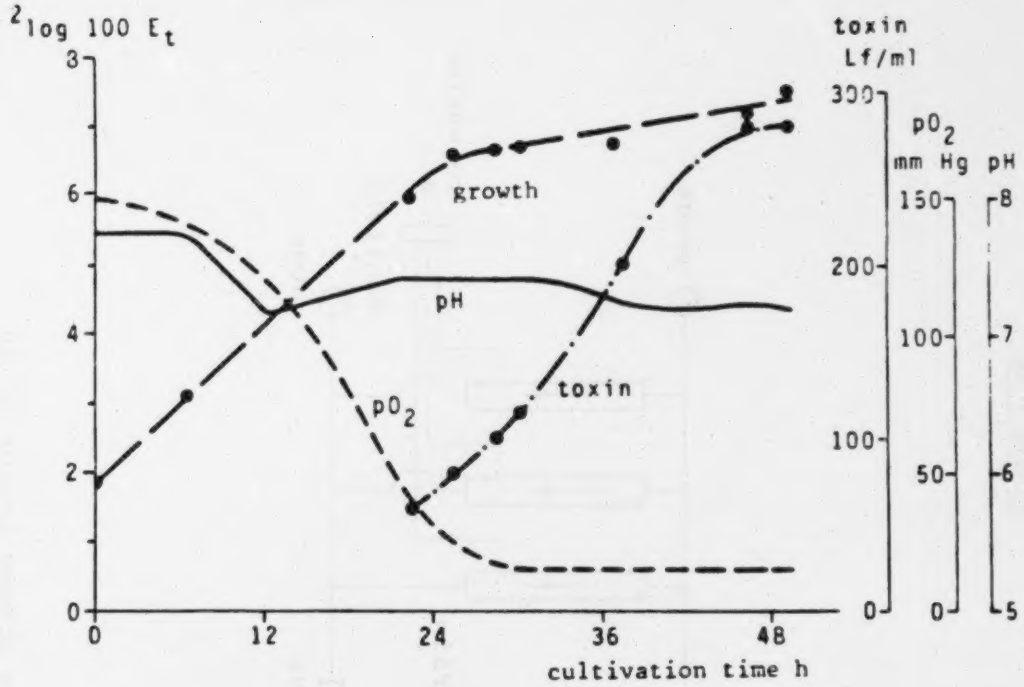
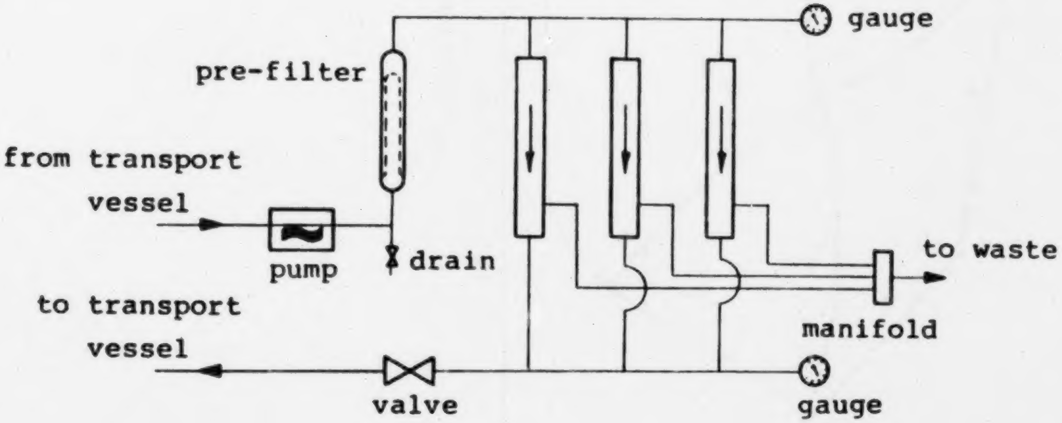
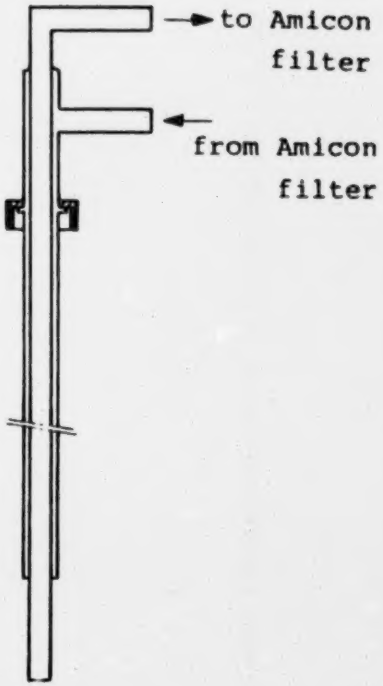


Figure 1.4B



Flow scheme Amicon DC 30
ultrafiltration apparatus



Double pipe system
for transport vessel

1.5 Preparation of purified tetanus toxoid

1.5.1 Introduction

A definition of purified tetanus toxoid as produced at the RIVM is:

> "Purified tetanus toxoid is a concentrated sterile solution of formalin detoxified and ammonium sulphate purified toxin of Clostridium tetani in a physiological saline solution"

The tetanus toxin is prepared in homogeneous culture in the Bilthoven Unit System at 140 or 350 l volume. Because Cl.tetani can form spores which are rather resistant to disinfection, WHO minimum requirements stipulate that special premises, equipment and precautions are used to manufacture tetanus toxin (ref. 24). All materials have to be disinfected before leaving the production unit and persons entering or leaving the unit have to change clothes. In our introduction we have argued that the production of tetanus toxin could be produced on a campaign basis sharing the same facilities and equipment as used for other bacterial vaccines, provided that strict measures for cleaning and disinfection are employed between the campaigns.

The culture medium is Müller-Miller medium with some slight modifications. Cl.tetani is an anearobic micro-organism and the cultivation has to be started under anearobic conditions. However, during the growth and lysis phase the culture may be gently mixed and flushed with air. There is even strong evidence that in an anaerobic atmosphere of pure nitrogen during the lysis phase the tetanus toxin released to the culture fluid is unstable (ref. 9). From the data presented in figure 1.5 it can be seen that cell lysis is completed after 6 to 7 days and corresponds with the maximum of toxin content in the culture fluid. The average yield, mainly depending on the batch of NZ-case, is around 65 Lf/ml. Detoxification of the filtered broth is carried out with 0.4% formaldehyde at 35 °C.

1.5.2 Strain

The strain of *Cl.tetani* used, is the Harvard strain no. 49205. It was obtained in lyophilized state from the Division of Laboratories and Research of the New York State Department of Health in Albany. For lyophilization the strain is grown on freshly prepared glucose beef heart broth, centrifuged and resuspended in skimmed milk. A gram-smear shows Gram-positive and negative rods of the same thickness, but different in length. It forms no spores in Müller-Miller media and glucose beef heart broth, but in less rich media spores may be formed. The strain ferments glucose, unlike most strains of *Cl.tetani*.

1.5.3 Cultivation

1.5.3.1 Media

The detailed composition of the production medium and preparation methods for stock solutions are given below.

One hundred liter of this medium, according to Müller-Miller, contains the following ingredients to be added to 80 l distilled water in the order given, at a temperature of about 55°C:

Glucose	1100 g
NaCl (p.a.)	250 g
Na2HPO4 (p.a.)	100 g
KH2PO4 (p.a.)	15 g
MgSO4.7H2O (p.a.)	15 g
FeSO4.7H2O (1% sol. in distilled water)	4 g
Cystine-HCl (10%)	250 ml
Tyrosine-HCl (10%)	500 ml
Uracil-HCl (2.5%)	1000 ml
Ca.panthothenate in ethanol 25%	100 mg
Thiamine " "	25 mg
Pyridoxin.HCl " "	25 mg
Riboflavin " "	25 mg
Biotin " "	250 mg

NaOH 5 N	400 ml
Beef heart infusion*	5 ml
NZ-case solution**	15.0-25.0 l

After adjusting the volume to 100 l the pH is adjusted with NaOH 5 N to pH=7.3. Sterilization is performed at 120^{o}C for 20 minutes.

*The beef heart infusion is prepared as follows:
Suspend 7.5 kg minced, defatted beef heart in 7.5 l distilled water. Bring rapidly to boiling and boil during two minutes. Filter through filter paper and store at 4^{o}C.

**Preparation of NZ-case:
Dissolve the amount of NZ-case needed (5% more than calculated, to account for filtration losses) in distilled water, to obtain a 10% w/v solution by heating in a stainless steel container. Cool down to 40^{o}C, dilute to the final volume and add 1.25 g charcoal per litre. Stir the solution for 20 minutes. Filter the solution through filter paper (Schut nr.V727 Ø 38.5). Recycle the first filtrate because some charcoal may have passed through the filters.

To determine the optimal concentration of NZ-case, normally between 15 and 25 g/l, each new batch is tested for toxin production on a 0.5 l scale.

1.5.3.2 Inoculum

A lyophilized culture is transferred into tubes with glucose beef heart broth and incubated at 35^{o}C for 24-48 hours in a anaerobic jar filled with hydrogen plus 10% carbon dioxide. The inoculum for a 150 l culture is made by inoculation of 300 ml production medium in a 500 ml bottle with 10 ml *Cl.tetani* grown in glucose beef heart broth and followed by incubation at 35^{o}C for 7 to 8 hours.

1.5.3.3 Culture

Prior to inoculation samples are drawn from the medium for extinction and pH measurement, and for sterility control on Tarozzi broth and nutrient agar. At 150 l cultivation scale the starting air flow rate is 2 l/min. and the Vibromixer amplitude 1.0 mm. After 16 resp. 24 hours the air flow rate is increased to 10 l/min. resp. 25 l/min. and the Vibromixer amplitude to 1.6 mm resp. 2.1 mm. The cultivation is stopped as soon as all cells have lysed, normally after 6 or 7 days. A typical graphical representation of a production culture is given in figure 1.5.

1.5.4 Downstream processing

Separation of the lysed cells and toxin containing culture fluid is performed by a so called "triple filtration". The broth is clarified in three sequential filtration steps: one coarse using a Seitz KO filter pads, followed by a medium filter, Seitz K5, followed by a 0.22 micron sterilizing filtration.

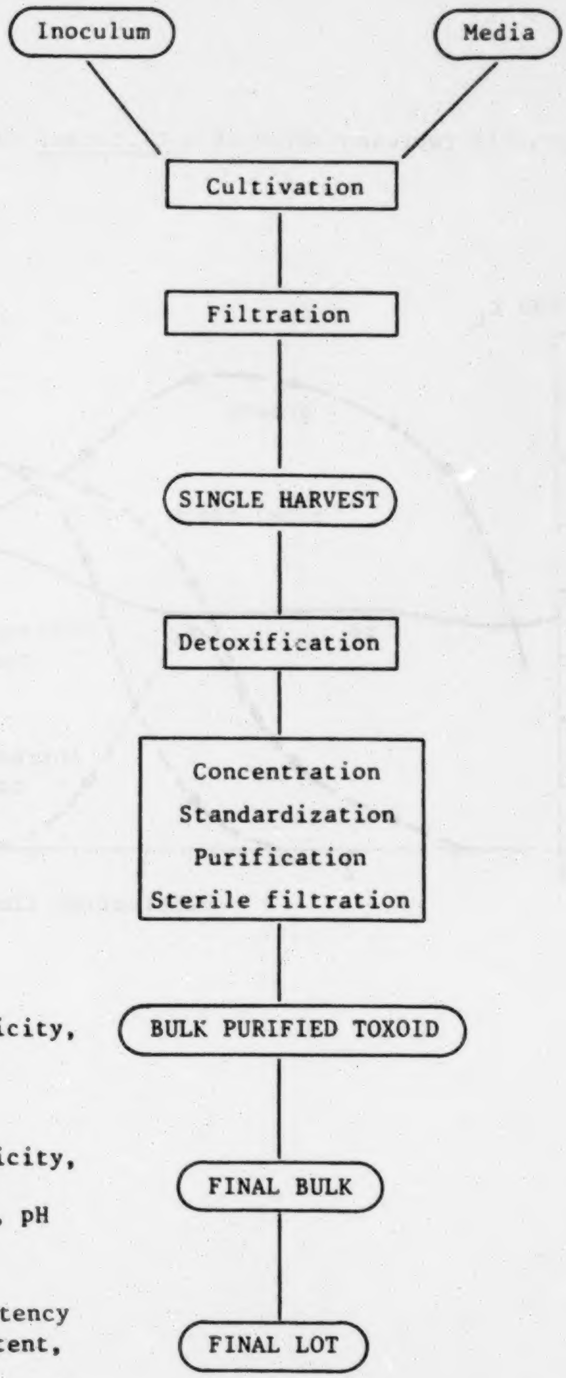
1.5.4.1 Detoxification

Direct after filtration 0.4% v.v. of a 40% w/v formaldehyde solution is added. After 2 hours mixing the toxin is passed through the wall to the incubator of 35^oC into a new sterile container to ensure that all toxin is well mixed with formalin. After seven weeks detoxification at 35^oC, a sample is taken for control of detoxification. Two weeks later, nine weeks at 35^oC, samples for sterility control, specific toxcicity and Lf determination are taken and the container awaiting release, is kept at 4^oC.

1.5.4.2 Purification

The method for purification is analogous to the method applied for diphtheria toxoid (see 1.4.4.3) except that for the pilot precipitation study 2.25, 2.75, 3.50, 4.25, 5.00 and 5.75 g $(NH_4)_2SO_4$ per 25 ml of toxoid solution are added.

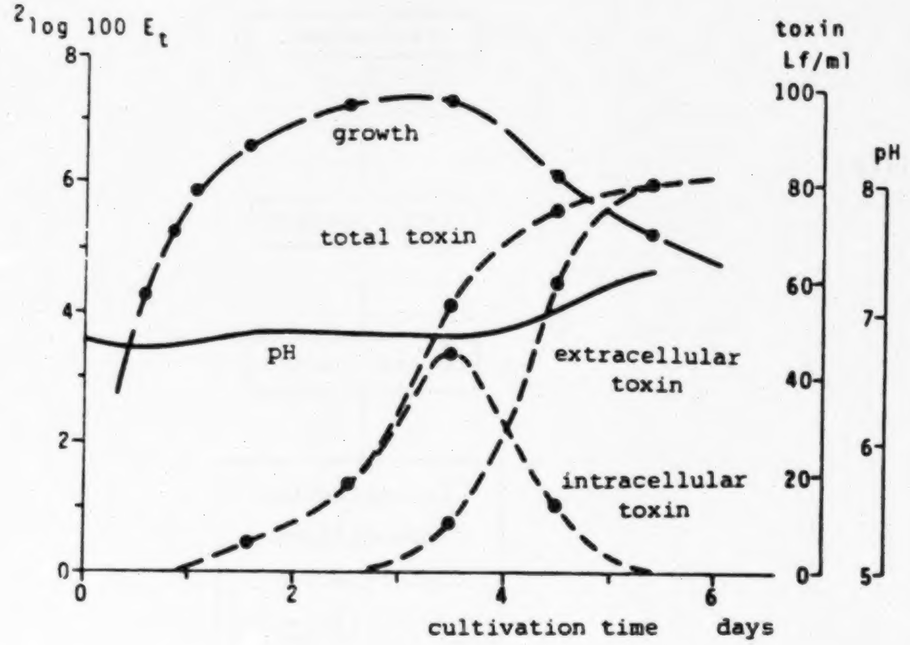
FLOW SHEET TETANUS TOXOID PRODUCTION (1.5)



CONTROLS	PROCESS
bacterial purity	Inoculum Media
bacterial purity pH Lf	Cultivation
	Filtration
sterility Lf Kf pH	SINGLE HARVEST
	Detoxification
	Concentration Standardization Purification Sterile filtration
sterility, specific toxicity, antigen content, purity	BULK PURIFIED TOXOID
sterility, specific toxicity, potency, residual free formaldehyde, stability, pH	FINAL BULK
identity, sterility, potency innocuity, adjuvant content, preservative content	FINAL LOT

Figure 1.5

Typical graphic representation of a Cl.tetani culture at 150 l scale



1.6 Preparation of inactivated poliomyelitis vaccine on VERO cells

1.6.1 Introduction

A flow chart of the preparation of Inactivated Poliomyelitis Vaccine is given at the end of this paragraph (figure 1.6). Starting from the Master seed cells stored in liquid nitrogen or in a mechanical freezer with a temperature of at least -90 °C, the Working Cell Bank (WCB) is produced by subcultivation of seed cells in monolayer respectively microcarrier culture. These cells are frozen and stored in liquid nitrogen respectively a -90 °C freezer for the initiation of the production cell cultures in microcarrier culture in Bilthoven Unit bioreactors equipped with control systems for temperature, pH, dissolved oxygen and stirrer speed. The production cell cultures are inoculated with one of the three types of poliovirus 1, 2 or 3 and harvested after 2 to 3 days. The virus harvests are clarified by filtration, concentrated by ultrafiltartion and purified by column chromatography on Sepharose CL6B and DEAE-Sephadex or -Sepharose. Concentrated, purified virus suspension is suspended in normal strength Medium 199 by addition of concentrated medium 199, filtered and then inactivated by addition of 40% w/v formaldehyde and incubation at 37 °C. The concentrated, purified monovalent vaccines are mixed at a ratio of respectively 40:8:32 D-antigen units to form a 10x concentrated trivalent bulk vaccine.

Hence the definition of the product is:

"Trivalent Inactivated Poliomyelitis Vaccine is a mixture of formalin inactivated poliomyelitis virus type 1, type 2 and type 3 in medium 199 at a concentration of 40:8:32 D-antigen units"

1.6.2 Materials and solutions

1.6.2.1 VERO cell culture medium

The media used for VERO cell cultivation are generally based on enriched Eagle's Minimum Essential Medium (MEM) such as DMEM or Iscove's medium supplemented with calf or bovine serum and an antibiotic solution. The medium can be bought on the market in powder or in concentrated form.

1.6.2.2 Virus cultivation medium

The medium used for virus multiplication is Medium 199 supplemented with an antibiotic solution. The same medium is used during inactivation. Medium 199 is usually bought from commercial firms and therefore its preparation is not described here.

1.6.2.3 Antibiotic solution (100x concentrated)

Streptomycin	7.5×10^6 IU
Neomycin	1.4×10^6 IU
Polymycin B sulphate	3.5×10^6 IU
Distilled water	to 1000 ml

1.6.2.4 0.025 M Sodium citrate solution

NaCl	8.0 g
KCl	0.2 g
Na2HPO4	1.0 g
KH2PO4	0.2 g
Trisodium citrate	6.5 g
Distilled water	to 1000 ml pH 7.3 ± 1

1.6.2.5 2.5% Trypsin solution (10x concentrated)

0.025 M sodium citrate buffer	900 ml
Antibiotics solution	100 ml
Glucose	20 g
Lactalbumin hydrolysate	20 g

1.6.2.6 Phosphate buffered saline (PBS)

NaCl	8.0 g
KCl	0.2 g
NA2HPO4	1.0 g
KH2PO4	0.2 g

Distilled water	to 1000 ml pH 7.3 ± 0.1

1.6.2.7 Phosphate buffer 0.04 M

Na_2HPO_4	1.0 g
KH_2PO_4	0.2 g
Distilled water	to 1000 ml pH 7.3 ± 0.1

1.6.2.8 Microcarriers

Cytodex 1, Pharmacia, Sweden

1.6.2.9 VERO-cells

VERO-cells may be obtained from the American Type Culture Collection at a passage level of about 130.

1.6.2.10 Seed viruses

Type	Strain	Source
poliovirus type 1	Mahoney	Received from Dr.Salk (1958)
poliovirus type 2	MEF-1	Received from Statens Serum Institut, Copenhagen, code 451 (1956)
poliovirus type 3	Saukett	Received from Statens Serum Institut, Copenhagen, code TV261 (1960/1961)

1.6.3 Description of procedures

The processes used at the RIVM to produce inactivated poliovaccine on monkey kidney cells has been described in detail in the literature (ref. 25, 26). There is no basic difference when VERO cells are used as substrate for virus multiplication.

The following gives a general review of the processes.

1.6.3.1 Cell cultivation

1.6.3.1.1 Culture methods

For small scale up to about 1 l cells are cultivated in standard monolayer cultures in Roux bottles. For large scale above a few liters the microcarrier culture system is used. In this system cells are cultivated in bioreactors which are controlled for temperature (37 °C), pH (7.2), dissolved oxygen (50% air saturation) and stirrer speed (50-150 rpm). Two to three gram Cytodex 1 is added to the culture medium as microcarriers. In order to achieve higher cell densities fresh medium is added either batchwise or preferably in a continuous perfusion system. In this way cell concentrations up to 3 x 10^6 cells can be achieved. In between each subculture cells are trypsinized from the carriers and subsequently added to the following bioreactor with fresh medium and carriers. Samples for control are taken as indicated in the flow chart.

1.6.3.1.2 Seed cells

Cells received from the ATCC or other sources are subcultivated in monolayer culture one or twice and frozen in aliquots of 10 to 20 x 10^6 cells in 1 ml medium plus 5% DMSO in liquid nitrogen.

1.6.3.1.3 Working Cell Bank (WCB)

Seed cells are subcultivated in monolayer culture and subsequently in microcarrier culture for the preparation of the WCB. A praticable subcultivation scheme is from 1 Roux bottle to 4 Roux bottles and then to 16 Roux bottles and on to a 3 l microcarrier culture, and then to a 40 l microcarrier culture. Cells from the 40 l microcarrier culture are trypsinized from the carriers and frozen in aliquots of 500 x 10^6 cells in 25 ml medium plus 5% DMSO in liquid nitrogen to form the WCB.

1.6.3.1.4 Production cell culture

Cells from the WCB are subcultured in microcarrier culture in bioreactors for the preparation of the production cell culture(s). The subcultivation scheme recommended is two ampoules of the WCB to 10 l microcarrier culture and thence to a 150 l microcarrier culture which can feed two 1000 l microcarrier cultures.

1.6.3.2 Virus cultivation

1.6.3.2.1 Preparation of Master- and working seed viruses

From the virus strains received a stock of Master-seed viruses is prepared by two subcultivations on monolayer cultures of VERO cells. Virus suspensions of both the first and second passage are dispensed in respectively 1 ml and 10 ml aliquots and stored at -70°C.

For the preparation of the working seed virus for production cultures the Master-seed virus of the second passage is subcultivated once more in a 20 to 40 l microcarrier culture of VERO cells. The virus suspension is dispensed in aliquots of 0.5 or 1 l and stored at -70°C.

Cell cultures used for the preparation of Master- and working seed viruses are controlled for sterility, extraneous agents, identity and tumorigenicity according to recommendations of WHO (ref. 11). The virus suspenions are controlled for sterility, $TCID_{50}$, extraneous agents and identity.

1.6.3.2.2 Virus production culture(s)

Prior to virus cultivation, samples are taken for control tests. A sample not less than 500 ml from each culture is taken for control on the presence of extraneous agents, mycoplasma and the first 5 successive production cultures from one batch of WCB cells for tumorigenicity (ref. 11).

The cell culture medium is removed, the cells are washed once with medium 199, resuspended in medium 199 and inoculated with one of three polioviruses.

The virus suspension is harvested 3 days after inoculation.

From the harvest samples are taken for testing as indicated in the flow chart (figure 1.6).

1.6.3.3 Processing virus harvests

1.6.3.3.1 Clarification

The virus harvest is clarified by filtration through a prefilter with the addition of a filter-aid and subsequently through a series of membrane filters. The final sterilising filter is a 0.2 um filter.

1.6.3.3.2 Concentration

The clarified virus harvest is concentrated by ultrafiltration through a Hollow Fiber cartridge system with a cut off of 10^5 molecular weight. The system is maintained in 2.5% formalin in distilled water for at least 2 days prior to a concentration run. The filters are rinsed repeatedly with sterile distilled water prior to use. The washing is such that formaldehyde is not detected in the final wash fluid. The virus is concentrated by recycling through the Hollow Fiber filters until a final volume of approximately 250 ml is achieved. The system is rinsed repeatedly with 0.04 M phosphate buffer, the rinses are concentrated and then added to the original concentrate. The total volume of the concentrate is approximately 1 1 from 1000 1 of harvest.

1.6.3.3.3 Purification

The concentrated virus harvests are purified by gel filtration on Sepharose CL6B and DEAE-Sephadex A-50 column chromatography. Gel filtration is performed on a Pharmacia K215/100 column packed with Sepharose CL6B to a volume of approximately 25 1. The column is maintained in 1% formalin in PBS for at least 4 days prior to a run. The formalin is removed by washing with approximately 60 1 of sterile 0.04 M phosphate buffer until the eluent is free of formaldehyde. The concentrate is applied to the column and eluted with sterile 0.04 M phosphate buffer. Based on absorbance patterns at 254 and 280 nm, the virus peak (approximately 4 l) is collected. The gel filtrate is then passed through a sterile column containing DEAE-Sephadex A-50, using 0.04 M phosphate buffer for elution. The final volume of the DEAE-fraction is approximately 5 l. At this point samples are taken for assessment of protein nitrogen content per D-antigen unit. The DEAE-fraction is diluted with concentrated Medium 199 (without added phosphate), glycine is added to a concentration of 0.5% and a sample is taken to determine D-antigen content. Following D-antigen determination, the material is diluted with Medium 199 without added phosphate and supplemented with 0.5% glycine such that the maximum D-antigen content per ml is 2000 for type 1, 1000 for type 2 and 1500 for type 3.

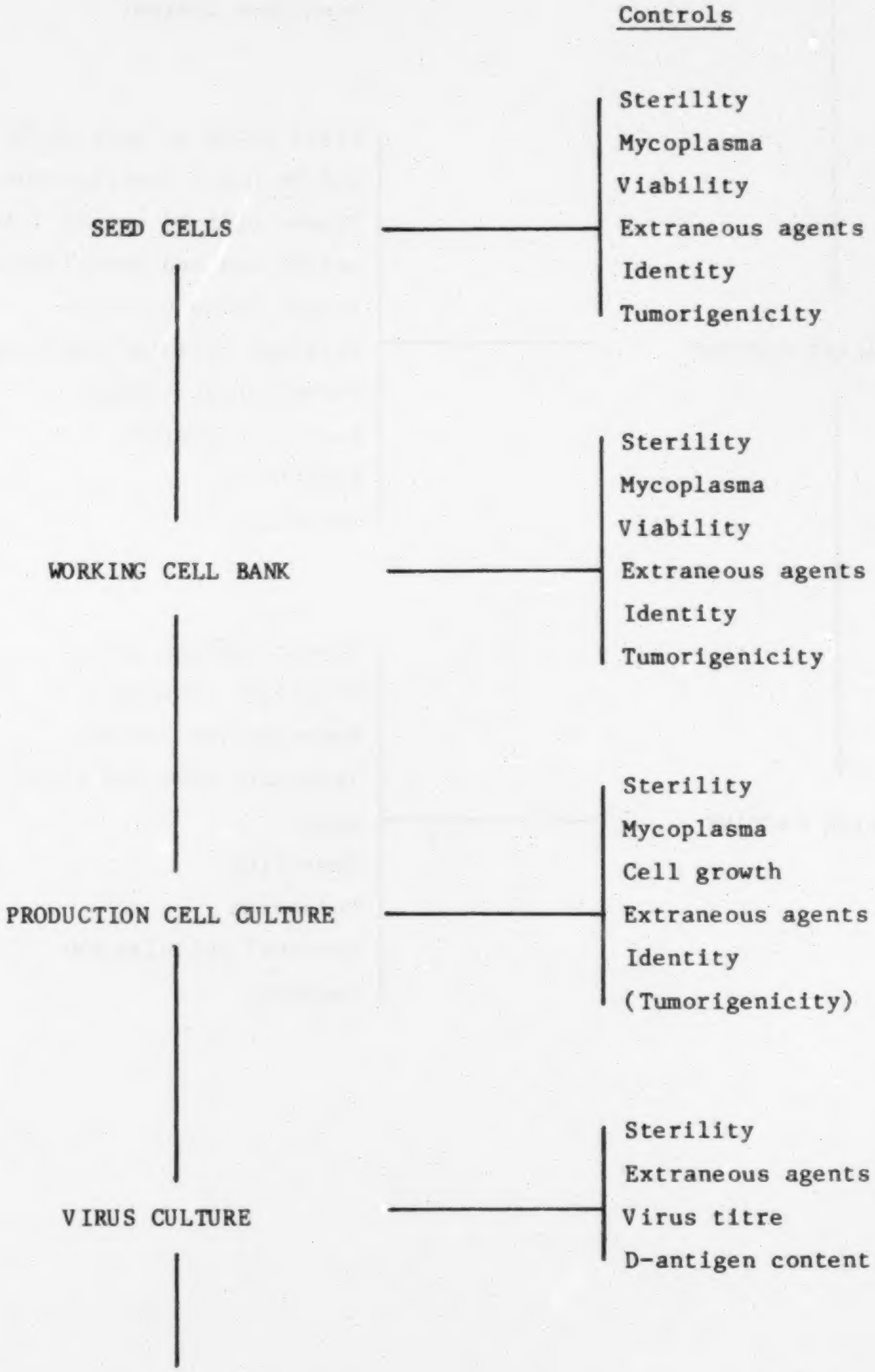
1.6.3.4 Inactivation

Within three days before the start of the inactivation process the purified virus suspension is filtered through a 0.2 µm sterile filter. Samples of the filtered virus suspensions are taken for sterility, identity, D-antigen, $TCID_{50}$ tests. 2.5 ml of a 3.5% formaldehyde solution is then added to each liter virus suspension (final dilution is 1:4000). The material is mixed thoroughly and incubated at 36-38 ^{o}C for 13 days at a pH of 6.9 ± 0.1. Samples are taken for virus titration at 0, 24, 48, 72 and 96 hours. Sodium bisulphite (0.14 ml of a 1:16 diluted 35% solution per 10 ml) is added to these samples for neutralization of the formaldehyde. The virus suspension is filtered through a 0.2 µm sterile filter 6 to 7 days after the start of the inactivation. The virus suspension is transferred into a new sterile bottle 9 to 10 days after the start of the inactivation. Samples representing 1500 doses each are taken on day 10 and 13 for testing on absence of live virus. At the completion of inactivation, samples are taken for sterility, D-antigen, serum protein, formaldehyde content and residual cellular DNA.

1.6.3.5 Mixing of the concentrated trivalent bulk

The monovalent vaccines are mixed to form a concentrated trivalent bulk such that the ratio of D-antigen for types 1, 2 and 3 is 40:8:32. Samples are then taken for toxicity, tissue culture safety (1500 doses total), serum content, D-antigen content and formaldehyde content. The concentrated trivalent vaccine is then filtered through a 0.2 µm sterile filter and samples are taken for *in vivo* potency, D-antigen content and sterility.

FLOW CHART OF THE PRODUCTION OF INACTIVATED POLIOMYELITIS VACCINE ON VERO CELLS



VIRUS HARVEST

- Sterility
- Virus titre
- Identity
- Protein nitrogen content
- D-antigen content

MONOVALENT VACCINE

- Virus titre at 0,24,48,72 and 96 hours inactivation
- Tissue culture safety 3 days before and end inactivation
- Bovine serum proteins
- Residual cellular DNA content
- Formaldehyde content
- D-antigen content
- Endotoxin
- Sterility

TRIVALENT VACCINE

- Tissue culture safety
- D-antigen content
- Formaldehyde content
- Innocuity mice and guinea pigs
- Sterility
- Endotoxin
- Residual cellular DNA content

1.7 Preparation of inactivated rabies vaccine on VERO cells

1.7.1 Introduction

A flow chart of the preparation of inactivated rabies vaccine on VERO cells is given at the end of this paragraph (figure 1.7). In principle it does not differ significantly from that for inactivated polio vaccine. The cell cultivation stage is exactly the same as at the production of inactivated polio vaccine. The production cell cultures are inoculated with seed virus in medium 199 containing 1% foetal calf serum. After an incubation of 5 to 7 days, cells are washed with medium 199 without serum and virus is harvested every 4 to 5 days up to 5 to 6 times. The virus harvests are clarified by filtration, concentrated by ultrafiltration and purified by column chromatography on DEAE-Sepharose CL6B.

The concentrated, purified virus suspension is inactivated by addition of β-propiolacton (BPL) at a concentration of 1/4000. After inactivation it is diluted to 6 IU on basis of an ELISA test with addition of 5% lactose and subsequently freeze-dried in amounts of 1 dose per vial.

Hence the definition of the product is:

"Inactivated Rabies Vaccine is freeze-dried BPL inactivated rabies virus in PBS with 5% lactose as stabilizer"

1.7.2 Materials and solutions

For the production of rabies virus the same media and cells (VERO cells) are used as for the production of poliovirus.

1.7.2.1 Seed viruses

The original PM strain used for brain tissue vaccine has been adapted to tissue culture at various laboratories. From the research carried out at the RIVM it might be concluded that most of them will grow well on VERO cells. However, most of these strains are not freely available and one has to come to an agreement with the laboratory concerned for the use of their strain.

1.7.3 Description of procedures

The process used at the RIVM to produce inactivated rabies vaccine on dog kidney cells has been described in literature (ref. 26, 26a). Basicly there is no difference when VERO cells are used as substrate for virus multiplication, except that the gel filtration on Sepharose CL6B is replaced by column chromatography on DEAE-Sepharose CL6B in order to remove the cellular DNA from the virus suspension.

The following gives a general review of the processes.

1.7.3.1 Cell cultivation

The cell cultivation procedures are similar to those used at the production of poliovirus.

1.7.3.2 Virus cultivation

1.7.3.2.1 Preparation of Master- and working seed viruses

The virus strain received for production are passed two times on monolayer cultures of VERO cells. The monolayer cultures are inoculated with the virus in medium 199 plus 1% foetal calf serum and incubated for 6 to 7 days at 35-36 ^{o}C. After this period cultures are frozen and thawed. Cell debris plus virus is harvested and the virus suspension of both the first and second passage are dispensed in aliquots of respectively 5 and 25 ml and stored at -70 ^{o}C. For the preparation of working seed virus for production cultures the second passage of the Master-seed virus is passed once more on VERO cells in a 20 l microcarrier culture. After cell growth the cell culture medium is removed and cells are incubated with 25 ml of virus suspension after dilution in medium 199 plus 1% foetal calf serum to 500 ml for 30 to 60 minutes. Medium 199 plus 1%

foetal calf serum is added up to the original 20 l. After an incubation period of 6 to 7 days, the supernatant and microcarriers plus cells are harvested. The microcarriers plus cells are frozen and thawed and cells are removed from the carriers by vigorously mixing with supernatant. The carriers are removed from this mixture and the virus suspension is dispensed in aliquots of 500 ml and stored at $-70^{o}C$.

Cell cultures used for the preparation of Master and working seed virus are controlled for sterility, extraneous agents, identity and tumorigenicity. The virus suspensions are controlled for sterility, LD_{50} in mice, extraneous agents and identity.

1.7.3.2.2 Virus production cultures

Prior to virus cultivation a sample of not less than 500 ml is taken from the production culture for control tests on the presence of extraneous agents, mycoplasma and for tumorigenicity at the first 5 production cultures from a new batch of the WCB. A second sample is taken for sterility control.

The cell culture medium is removed and the cells are inoculated with seed virus in medium 199 plus 1% foetal calf serum according to the procedure described under 1.5.3.2.2. After 6 days the culture fluid is harvested and discarded after disinfection. Microcarriers with cells are washed thoroughly with medium 199 without serum and resuspended in this medium. From this culture every 4 to 5 days virus is harvested repeatedly until the cell number is dropped to 150 to 200 cells per ml. Generally 4 to 6 harvests may be obtained. Virus harvests are stored at $4^{o}C$ untill the last harvest has been taken. From the harvests equal samples are taken and mixed together for control tests as indicated in the flow chart.

1.7.3.3 Processing virus harvests

1.7.3.3.1 Clarification

The virus harvests are clarified by filtration through a membrane filter of about 1 μm.

1.7.3.3.2 Concentration

The clarified virus suspension is concentrated by ultrafiltration through a Hollow Fiber cartridge system with a cut off of one million MW according to the procedure described under paragraph 1.5.3.2.2. PBS instead of 0.04 M phosphate buffer is used for rinsing the system.

1.7.3.3.3 Purification

The concentrated virus harvest is purified by passing through a sterile column with DEAE-Sepahrose CL6B in PBS. From the purified virus suspension samples are taken for control tests as indicated in the flow chart.

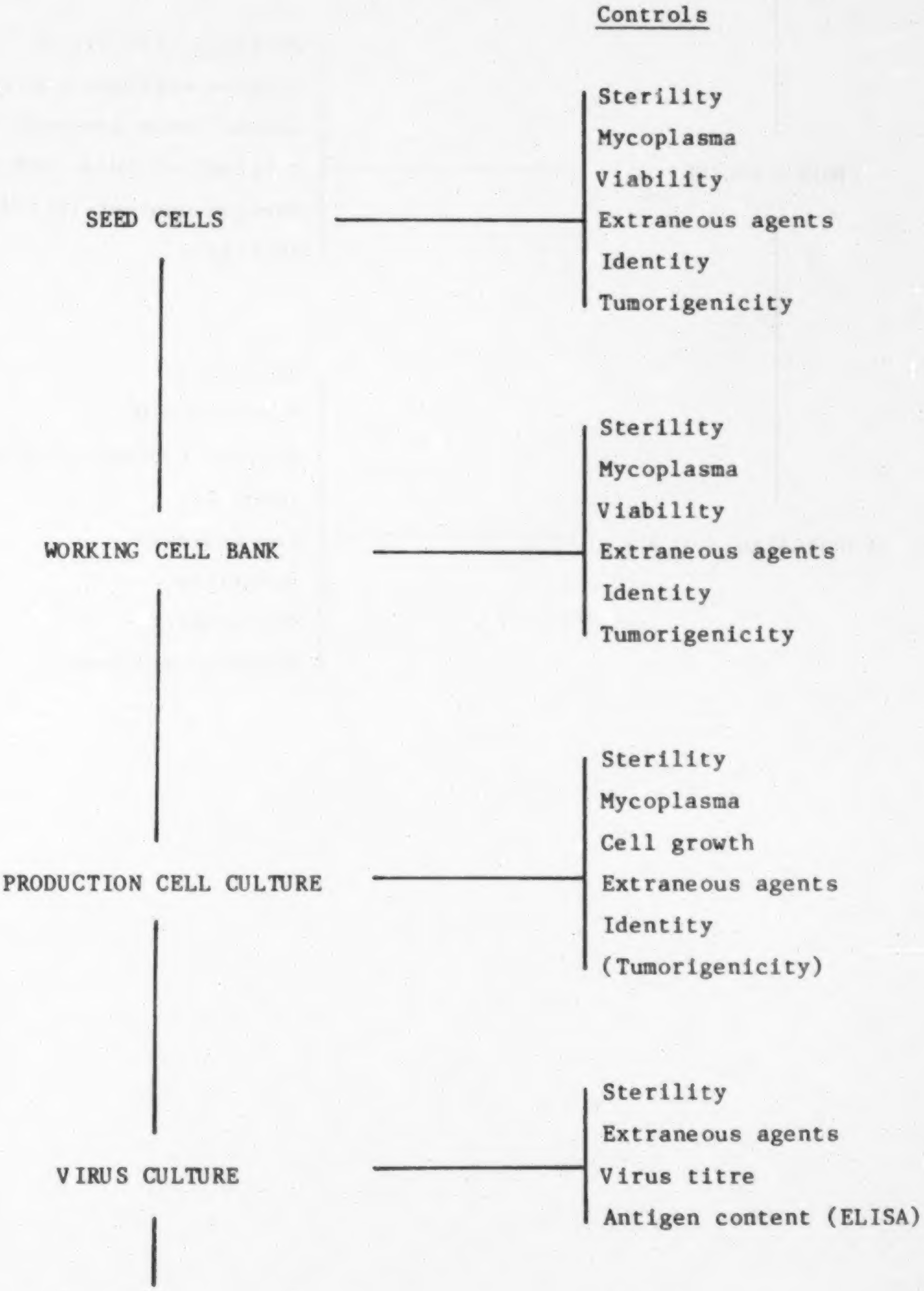
1.7.3.3.4 Inactivation

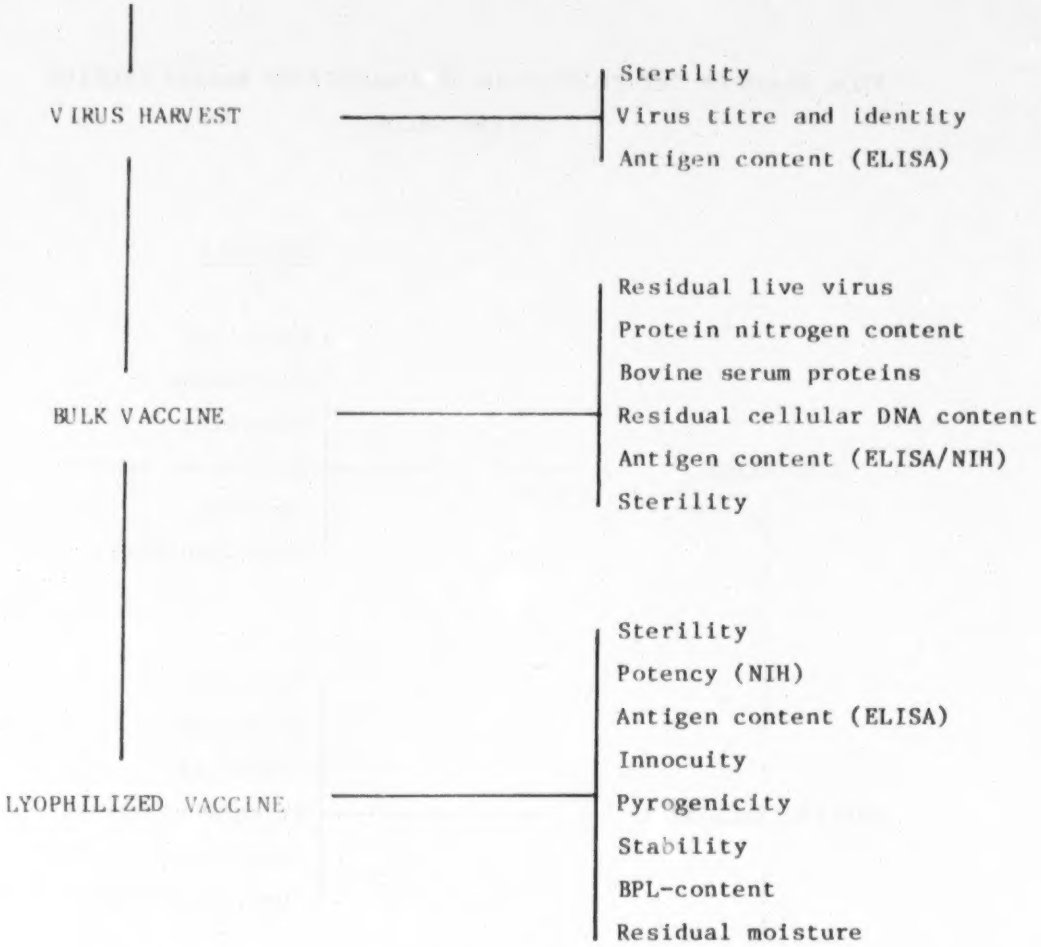
The purified virus suspension is inactivated by the addition of BPL to a concentration of 1/4000 and overnight incubation at $4^{o}C$ and subsequently two hours at $37^{o}C$. Samples for control tests are taken as indicated in the flow chart.

1.7.3.3.5 Freeze drying

Prior to freeze drying the inactivated vaccine is stored for 3 weeks at $4^{o}C$, diluted with PBS to about 6 IU on basis of ELISA with addition of 5% lactose and dispensed in vials of 1 ml. After freeze drying samples are taken for control tests as indicated in the flow chart.

FLOW CHART OF THE PRODUCTION OF INACTIVATED RABIES VACCINE ON VERO CELLS




VIRUS HARVEST
Sterility
Virus titre and identity
Antigen content (ELISA)
BULK VACCINE
Residual live virus
Protein nitrogen content
Bovine serum proteins
Residual cellular DNA content
Antigen content (ELISA/NIH)
Sterility
LYOPHILIZED VACCINE
Sterility
Potency (NIH)
Antigen content (ELISA)
Innocuity
Pyrogenicity
Stability
BPL-content
Residual moisture

1.8 Preparation of live measles vaccine

1.8.1 Introduction

A flow chart of the preparation of live measles vaccine on primary chick fibroblasts is given at the end of this paragraph (figure 1.8). The eggs for the preparation of the cell culture should be obtained from a cloned flock of chickens regularly monitored for the presence of infectious agents. The eggs are embryonated by incubation at 37°C for 10 to 11 days. Embryo's are trypsinized and cultivated in standard monolayer or roller tube glass vessels. After 3 to 4 days cultures are inoculated with virus in fresh medium and incubated at 33°C for another 4 days. Virus infected cell cultures are thoroughly washed with PBS to remove of bovine serum proteins and incubated in medium 199 at 33°C. After 3 to 5 and subsequently every 1 or 2 days virus is harvested. In general up to 2 to 5 harvests may be obtained until monolayers are destroyed by the virus. Virus harvests are pooled and after clarification by filtration stored at -70°C. Before freeze drying a stabilizer is added in order to protect the virus against temperature degradation.

Hence the definition of the product is:
"Live measles vaccine contains a freeze dried suspension of measles virus in medium 199 to which a special stabilizer is added."

1.8.2 Materials and solutions

1.8.2.1 Eggs

Eggs are derived from a chicken colony regularly monitored for infectious agents (see measles control, Chapter 2).

1.8.2.2 Cell culture medium

Eagle's Minimum Essential Medium (MEM) or Medium 199 supplemented with 3 to 10% foetal calf serum are generally used for cell cultivation.

1.8.2.3 Virus cultivation medium

The medium for virus cultivation is Medium 199.

1.8.2.4 Trypsine solution

0.25% Trypsine in PBS sterilized by filtration

1.8.2.5 Phosphate buffered saline (PBS)

NaCl	8.0 g
KCl	0.2 g
Na_2HPO_4	1.0 g
KH_2PO_4	0.2 g
distilled water	to 1000 ml pH 7.3 ± 0.1

This solution may be sterilized either by filtration or autoclavation.

1.8.2.6 Seed viruses

Attenuated measles virus strains are used for production of live measles virus vaccine. Moraten or Scharwtz strain are most commonly used. These strains are not freely available.

1.8.3 Description of procedures

1.8.3.1 Cell cultivation

Fertilized eggs are incubated at 37°C for 10 to 11 days. The embryo's are aseptically taken out and after removal of the head the embryo tissue is dispensed in a single cell suspension by treatment with a 0.25% trypsine solution. The cell suspension is centrifuged for removal of the trypsin solution and resuspended in the cell culture medium at a concentration of 200×10^3 cells per ml. The cell suspension is seeded in flat glass bottles (Roux or Povitsky bottles) or roller tubes and incubated at 37°C for 3 to 4 days until a closed monolayer is formed.

1.8.3.2 Virus cultivation

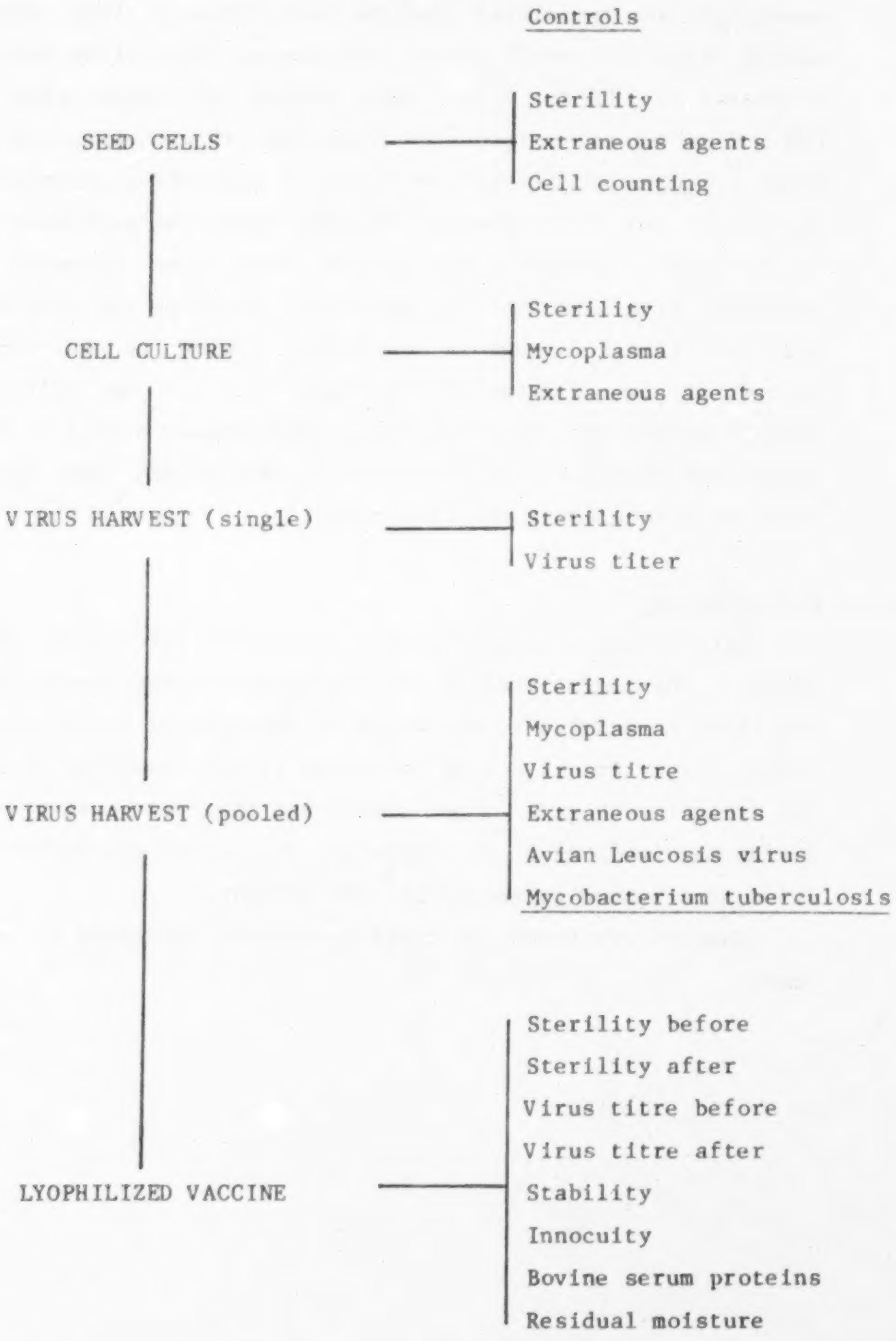
Before virus inoculation an appropriate number of cultures are taken for control. The cell culture medium is removed and monolayers are inoculated with the seed virus in virus cultivation medium plus 1 to 5% foetal calf serum. Cell/virus cultures are incubated at 33°C for 4 to 5 days, washed thoroughly with sterile PBS and subsequently incubated in medium 199 without serum at 33°C. After 4-5 days and further every 1 to 2 days virus suspensions are harvested and fresh medium 199 added until the monolayer of cells is destroyed. Generally two to five serum free harvests may be obtained from one set of cultures. Harvests are stored at +4°C until the latest harvest is obtained, pooled to one batch and clarified by filtration through a 1.5 um filter or by centrifugation and stored at -70°C until freeze drying. From the pooled and clarified virus suspension samples are taken for control tests as indicated in the flow chart.

1.8.3.3 Freeze drying

After thawing an appropriate stabilizer for freeze drying is added. The composition of the stabilizer is not freely available. The virus suspension is dispensed in ampoules or vials and freeze dried. After freeze drying the virus titers should be at least 10^3 PFU after 1 week at 37°C and should not have lost more than 1 PFU during this week at 37°C. Therefore in general the virus is freeze dried at a concentration of 10^4 PFU or more.

Samples are taken for control tests as indicated in the flow chart.

FLOW CHART OF THE PREPARATION OF LIVE MEASLES VACCINE



1.9 Preparation of final vaccines

1.9.1 Introduction

The preparation of the final vaccines for rabies and measles are already described in paragraph 1.7 and 1.8. For these freeze-dried vaccines only sterile water for injection has to be made available for reconstitution.

In this paragraph the preparation of:

a. Adsorbed tetanus vaccine
b. Plain inactivated polio vaccine
c. DPT-polio vaccine

will be described.

1.9.2 Preparation of adsorbed tetanus vaccine

1.9.2.1 Composition per ml

Tetanus toxoid	10 Lf
$AlPO_4$	3.0 mg
NaCl	9.0 mg
merthiolate	100 g
dose	0.5 ml

1.9.2.2 Sterile ingredients

Tetanus toxoid	1000 Lf/ml
Na_3PO_4	0.13 mol/l
$AlCl_3$	1 mol/l
NaCl	25% m/v
Na_2CO_3	20% m/v
merthiolate	1% m/v
distilled water	

1.9.2.3 Procedure for the preparation of 300 l

Fill into a weighed 300 l mixing vessel, equipped with pH electrode, 1000 g NaCl (as a 25% solution) and 195 kg distilled water. Sterilize at 120 °C for 1 h, let cool down, and make up to 200 kg with sterile distilled water. Homogenize by mixing for 15 minutes with a Vibromixer, and take a sample for determination of the chloride concentration. Calculate the chloride content of the mixing vessel (A gram). Add the merthiolate solution and the calculated volume of aluminium chloride solution to contain 7.38 mol $AlCl_3$. From the latter 1300 g NaCl will be formed. Calculate the missing quantity of NaCl (2700-A-1300 g), add this quantity as a calculated volume of sterile 25% NaCl solution, and bring the contents of the vessel to 238 kg with sterile distilled water. After homogenizing the contents of the mixing vessel, add with continuous mixing 3000 ml tetanus toxoid, and subsequently in c. 30 min 59 kg of Na_3PO_4 solution. Raise the pH with Na_2CO_3 solution to 6.00, and continue mixing for 2 h.

Take samples for sterility, innocuity, specific toxicity and potency tests, for determination of the concentration of free and bound aluminium, free phosphate, chloride and methiolate, and a spare sample of c. 250 ml.

After filling samples are taken for sterility control, $AlPO_4$ determination, identity (Lf determination) and general safety.

1.9.3 Preparation of plain polio vaccine

1.9.3.1 Composition per ml

Poliovaccine type 1	40 DU
type 2	8 DU
type 3	32 DU
2-phenoxyethanol	5 mg
formaldehyde	25 µg
Medium 199	1 ml
Dose	1.0 ml

1.9.3.2 Sterile ingredients

Concentrated trivalent inactivated polio vaccine	type 1	400 DU/ml
	type 2	80 DU/ml
	type 3	320 DU/ml

2-phenoxyethanol
formaldehyde solution
Medium 199

1.9.3.3 Procedure for the preparation of the final vaccine

The concentrated trivalent bulk is diluted with Medium 199 to a D-antigen concentration of 40:8:32 units per dose. 2-phenoxyethanol is added to the final bulk to a concentration of 0.5% and formaldehyde is added to a final concentration of 25 μg per ml. Before filling samples are taken from the final bulk for sterility, 2-phenoxyethanol content, formaldehyde content, D-antigen content and *in vivo* potency. The final bulk is filled in glass ampoules or vials. After filling, samples are taken for testing of sterility, D-antigen content (identity) and general safety.

1.9.4 Preparation of DPT-polio vaccine

1.9.4.1 Composition per ml

Diphtheria toxoid	15 Lf
Pertussis	16 IOU
Tetanus toxoid	10 Lf
Polio type 1	40 DU
type 2	8 DU
type 3	32 DU
AlPO4	2.0 mg
Sodium chloride	9.0 mg
2-phenoxyethanol	5.0 mg
formaldehyde	25.0 μg
dose	1.0 ml

1.9.4.2 Sterile ingredients

Diphtheria toxoid		1125 Lf/ml
Pertussis suspension		200 IOU
Tetanus toxoid		1000 Lf/ml
Concentrated trivalent polio	type 1	400 DU/ml
	type 2	80 DU/ml
	type 3	320 DU/ml
Na3PO4		0.13 mol/ml
AlCl3		1 mol/l
NaCl		25% m/v
HCl		2 mol/l
Na2CO3		20% m/v
2-phenoxyethanol		pure
formaldehyde		2% m/v
distilled water		

Concentrated dilution fluid:

NaCl	74.2 g
KCl	4.0 g
CaCl2.2HO	2.0 g
MgSO4.7H2O	2.0 g
Phenol red	200 mg
Distilled water	1000 ml
pH (with HCl)	5.0

1.9.4.3 Procedure for the preparation of 300 l

Fill into the weighed 300 l mixing vessel, equipped with pH electrode, 180 kg distilled water and 20 kg concentrated diluent for DPT-polio vaccine. Bring the pH to 5.0 and sterilize at 120^{o}C for 1 h. Add after cooling sterile distilled water to 200 kg. Take a sample for checking of the pH electrode. Press c. 20 l liquid from the mixing vessel into a sterile bottle, which remains connected to the mixing vessel. Add while mixing, the calculated amount of AlCl3 solution to contain 4.93 mol AlCl3. Check the pH (should be around 3.8), and add in c. 15 min with continuous mixing Na3PO4 solution until the pH reaches 4.5 (c. 40 kg should be added). Raise the pH with Na2CO3 solution to 5.00. Add, while mixing, 1500 g 2-phenoxyethanol and the calculated amount of formaldehyde solution to contain (7.5-p) g formaldehyde, where p is the formaldehyde content of the concentrated poliovaccine (c. 2.4 g). Then add, while mixing, subsequently:

- 30 l concentrated trivalent polio vaccine
- 4000 ml diphtheria toxoid
- 3000 ml tetanus toxoid

Correct the pH to 5.00, and continue mixing overnight. Bring the pH to 7.05 with Na2CO3 solution. Add, while mixing, 24 l concentrated pertussis vaccine. Mix well, and take a sample for determination of the chloride concentration. Calculate the NaCl content per l and adjust to 0.9 g/l with the 25% NaCl solution or distilled water. Correct the pH if necessary to 7.05 ± 0.05, and bring the weight to 300 kg with part of the diluent in the B-20 bottle. Continue mixing overnight.

Take samples for sterility, innocuity, specific toxicity, and potency (diphtheria, pertussis, tetanus, polio) tests, for determination of the concentration of free and bound aluminium, free phosphate, chloride, 2-phenoxyethanol, formaldehyde and polio D-antigen, and a spare sample of 250 ml.

After filling samples are taken for sterility control, AlPO4 determination, general safety and identity (Lf determination for diphtheria and tetanus, agglutination for pertussis and D-antigen determination for polio type 1, 2 and 3).

2. QUALITY CONTROL OF VACCINES

2.1. General

During the manufacture of a vaccine the facilities, equipment, staff and product at the key stages of manufacture have to be monitored by staff who are independent of production (ref. 27, 28, 29). Thus, the Head of Quality Assurance reports directly to the Head of the Institute and not to the Head of the Production Unit.

There are four main functions:

1. Monitoring the production environment to ensure air flows and filters are properly installed and functioning. Monitoring sterile areas for the bioburden of micro-organisms
2. Quality control testing: the taking and storage of test samples. The performance of the appropriate tests of quality of the product according to WHO recommendations or those of the relevant National Control Authority. Tests on all materials used in production are required, although the testing of some chemicals can be done on the basis of identity and inspection of analytical specification provided by suppliers.
3. Documentation:
   a) Ensuring that proper standing operating procedures exist for production and proper records are kept.
   b) Drawing-up and maintaining reports of test procedures and establishing limits for pass or fail.
   c) Preparation and inspection of production batch protocols dealing with production and testing.
   d) Submission of protocols to the National Control Authorities.
   e) Maintain records of production for reference.
4. The staff of Quality Assurance need to inspect the production facilities to ensure that the environmental conditions and standing operating procedures are being adhered to in every detail.

   They are also concerned with receiving and recording reports of adverse reaction and reporting these to the National Control

Authority. Where necessary records of vaccine tests involved will be inspected and extra testing initiated.

In addition to a Control Laboratory within the Institution making vaccines a National Control Laboratory may be established. [1]

To achieve effective control of quality the following rules apply:

a. Adequate facilities and staff should be available for sampling, inspection and testing of starting materials, packaging materials, intermediate, bulk and finished products and where appropriate, for determining environmental quality.
b. Samples of starting materials, packaging materials, intermediate products, bulk products and finished products should only be taken by personnel and using methods approved by the person responsible for Quality Control.
c. Results of the inspection and testing of materials and intermediate, bulk or finished products should be formally assessed (or a person designated by him) before products are

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[1] According to WHO recommendations there should be also in the countries a different and independant type of quality control, the National Control Authority, which generally runs a national control laboratory. This is a completely independent body, authorized to inspect production establishments in the country, to retest vaccine - produced at home or imported - if this is considered desirable, and whose approval is required before vaccines can be used in the field. It is important that every country should have such an authority, although the extent of its activities depends on the local situation. They may range from a simple inspection of submitted protocols to a fully equipped laboratory where each vaccine is retested. For further details see the relevant WHO document (ref. 23). Furthermore the national control agency should preferably be in a position to measure antibody responses to the vaccines, aiming at the local child population to ensure that they are effective. This report will focus only on quality control related to production.

released for sale or supply. Product assessment should include a review and evaluation of relevant manufacturing (including packaging) documentation.

d. Sufficient reference samples of starting materials and products should be retained (the latter, where possible, in the final package) to permit future examination if necessary.

The following section on quality control will concern only building facilities, equipment and staff needed for control tests on the 7 bacterial and viral vaccines dealt with in this respect. The facilities described are suitable for performing tests in accordance with requirements laid down by international agencies, in particular WHO.

It should be clear that the description of the quality control laboratory - staffing, equipment, facilities, etc. - is based on a fully comprehensive laboratory, where at least all seven vaccines, discussed in this document, have to be controlled. It will need modification if only some of the vaccines have to be tested, e.g. only bacterial vaccines. In that event the quality control laboratory can be much more limited in scope.

According to WHO recommandations (ref. 28) and to GMP rules (ref. 27) the quality control department must function separately from the production department, to enable independent judgment of the safety and efficacy of each batch of vaccine. Reports of the control department must be presented directly to the director of the whole vaccine production and control facility and/or to the national control authority. Although working areas, equipment and personnel of the control department must be separated from those of the production department, they can be housed in one building as long as sufficient physical barriers exist. The facilities of the control department should be at least equal in quality to those of the production facility.

In many cases - especially in potency testing - it is necessary to use certain standards or reference preparations, which are internationally accepted. WHO has established such standards or reference preparations for many vaccines and other biological products. A full list, which is revised from time to time, is available from WHO (ref.30). Additions and changes between editions of the list are always published in the yearly report of the Expert Committee on Biological Standardization (WHO Technical Report Series). The preparations can be obtained free of charge from an International Laboratory for Biological Standards. Which laboratory has to be contacted for a particular preparation can be found in the list (ref.30). International reference preparations, made available in very small quantities, are not intended for routine use but for calibration of national or laboratory standards for routine use. If a control laboratory does not have the means to calibrate such a house standard, it should contact an experienced laboratory and request help. Details of the production and calibration of local or national reference preparations can be found in WHO Guidelines (ref.31).

2.2. Summary of control tests required for vaccines

This survey of tests is restricted to the human vaccines, discussed in this document (the bacterial vaccines BCG and DPT and the viral vaccines against poliomyelitis (inactivated), measles and rabies).

Moreover only the fundamentals of the tests are mentioned and the essential elements to perform the tests in the laboratory (special equipment, use of animals, cell cultures etc.). Full details have been published, especially in several WHO documents (Requirements for Biological substances and Manuals, ref.11, 24, 28, 30, 32-37), but also in other internationally accepted publications (e.g. European Pharmacopeia, American FDA Regulations and national requirements in other countries). We shall refer here mainly to the relevant WHO publications.

Control tests required for BCG vaccine

1. Source Materials

1.1 Seed lot : tests for the absence of virulent mycobacteria (10 guinea pigs), identity, contaminating microorganisms (ref.36,38)

2. Final bulk : tests for contaminating microorganisms, absence of virulent mycobacteria (in guinea pigs, minimal 6, 6 weeks), total bacterial content (dry weight or opacity) (ref.36,38)

3. Final lot : tests for contaminating microorganisms, total bacterial content, number of culturable particles, stability, production consistency (ref.36,38)

Control tests required for Diphtheria toxoid

1. Source materials

1.1 Seed lot : control of culture characteristics (ref.24,33,38)

1.2 Culture medium: see ref.33

2. Single harvest : tests for bacterial purity, toxin concentration (Lf by flocculation with a standardised appropriate serum) (ref.24,33,38)

3. Bulk purified toxoid : tests for sterility (normal sterility test, ref.32,33,38), specific toxicity (5 guinea pigs, 6 weeks,) (ref.24a,33), antigenic purity by determination of Lf and protein nitrogen (ref.24a,33)

4. Final Bulk : tests for sterility (ref.32,33,38,) specific toxicity (5 guinea pigs, 6 weeks), potency (details see ref.24a, at least about 50 guinea pigs, in comparison with reference preparation, challenge with toxin; ref.24a,33,38). A method by immunizing mice, followed by blood antitoxin titration in cell culture, is recently accepted by the National Control Authority in The Netherlands. Residual free formaldehyde (ref.24a,27). Stability test (ref.24a,27). Measurement of pH.

5. Final product : tests for identity (by flocculation) (ref.24a,33,38), sterility (ref.32,33,38), potency (if not done in final bulk, ref. 24a), innocuity (injection of 2 guinea pigs and 5 mice, 1 week, ref.24a,33), adjuvant content (ref.24a), preservative content (ref.24a), pH.

Control tests required for Tetanus Toxoid

1. Source materials

1.1 Seed lot : control of culture characteristics (ref.24b,33,38)

1.2 Culture medium: see ref.33

2. Single harvests: tests for bacterial purity, toxin concentration (Lf by flocculation with suitable serum) (ref.24b,33,38)

3. Bulk purified toxoid : tests for sterility (normal sterility test, ref.32,33,38), specific toxicity (5 guinea pigs, 3 weeks) (ref.24b,33), antigenic purity by determination of Lf and protein nitrogen (ref.24b,33)

4. Final bulk : tests for sterility (ref.32,33,38), specific toxicity (5 guinea pigs, 3 weeks) (ref.24b,33), potency (details see ref.24b, at least about 50 guinea pigs or mice, in comparison with a reference preparation, challenge with toxin; ref.24b,33,38), residual free formaldehyde (ref.24b,33), stability (ref.24b,33), pH

5. Final product : tests for identity (by flocculation) (ref.24b,33,38), sterility (ref.32,33,38), potency (if not done in final bulk, ref.24b), innocuity (injection of 2 guinea pigs and 5 mice, 1 week, ref.24b,33), adjuvant content (ref.24b), preservative content (ref.24b), pH

Control tests required for pertussis vaccine

1. Source materials

1.1 Strains : see ref.33

1.2 Seed lot : control of culture characteristics (see ref.33)

1.3 Culture medium: see ref.24c

2. Single harvests: tests for bacterial purity and opacity (ref.24c,33)

3. Final bulk : tests for sterility (normal sterility tests, ref.32,33,38), toxicity (usually in mice, mouse weight gain test, 7 days, ref.24c,33,38), potency (mouse protection test, at least 60 mice + 60 for reference preparation, challenge with bacterial suspension, ref.24c,33,38), stability (ref.24c,33), pH

4. Final Product : tests for identity (agglutination with monospecific antisera, ref.24c,33,38), sterility (ref.32,33,38), potency (if not done in final bulk), innocuity (5 mice and 2 guinea pigs, 7 days, ref.24c,33), adjuvant content (ref. 24c), preservative content (ref.24c), pH

Control tests required for combined vaccines containing D,T and/or P

Tests for potency, specific toxicity, innocuity, sterility as described for each of the components present. Identity, flocculation of toxoids after centrifugation of the pertussis organisms. For details see ref.33.

Control tests required for inactivated poliomyelitis vaccine at various stages of the production process (ref.11)

1. Source materials

1.1. Virus seed lot : seed lots shall have been shown to be free from extraneous agents including viruses, mycoplasma, bacteria and fungi (ref.32).

1.2 Cell bank (continuous cell lines, VERO cells) : cells of the manufacturers working cell bank (MWCB) shall have been characterized with respect to their genealogy, karyology, growth characteristics, including tumorigenicity testing, and viability during storage. They shall have been shown by tests in animals, eggs and tissue cultures to be free from detectable extraneous agents and to be free from bacteria, fungi and mycoplasma (ref. 11, Annex 2, Part C)

1.3 Bovine serum : bovine serum used for cell culture propagation shall have been tested to show freedom from bovine viruses (ref. 40, Annex 3, Appendix 2) and from bacteria, fungi and mycoplasma.

1.4 Trypsin : trypsin used in cell culture propagation shall have been tested (ref. 40, Annex 3, Appendix 1) to show freedom from (porcine) viruses and bacteria, fungi and mycoplasma.

2. Cell culture (production) :
   - bacterial and mycotic sterility
   - mycoplasma
   - cell growth (nuclei counting)
   - control cell cultures of the production culture shall be tested for extraneous hemadsorbing and cytopathogenic viruses (2 weeks)
   - supernatant fluids of the control cell cultures shall be tested for extraneous agents in human cells, another sensitive cell system and in the same type of cells as used for vaccine production (4 weeks)
   - control cells shall be tested for tumorigenicity (ref. 11, Annex 2, Part C)

3. Virus culture (pool before processing) :
   - bacterial and mycotic sterility
   - extraneous agents in tissue culture (4 weeks)
   - virus titre
   - D-antigen concentration

4. Virus harvest (monovalent pool after filtration, concentration and purification) :
   - bacterial and mycotic sterility
   - virus titre and identity
   - D-antigen concentration (gel diffusion or ELISA)
   - protein nitrogen concentration

5. Inactivation process:
   - rate of inactivation (in tissue culture)

6. Monovalent vaccine :
   - tests in tissue culture for residual live poliovirus on a sample taken 3 days before the end of inactivation and on a sample taken at the end of the inactivation process (5 weeks)
   - bacterial and mycotic sterility

- residual cellular DNA and RNA
- D-antigen content (gel diffusion or ELISA)
- formaldehyde content
- endotoxin
- animal (bovine) serum proteins (counter-current electrophoresis)

7. Trivalent vaccine (bulk) :
   - test in tissue culture for residual live poliovirus (5 weeks)
   - bacterial and mycotic sterility
   - D-antigen concentration
   - formaldehyde content
   - innocuity
   - endotoxin

8. Final product (trivalent plain vaccine or combined vaccine) :
   - identity (potency test may be used)
   - potency tests *in vivo* (in rats or other suitable animal species) (4 weeks) and *in vitro* (D-antigen test by gel diffusion or ELISA or other suitable tests)
   - bacterial and mycotic sterility
   - innocuity (in mice and guinea pigs) (10 days)

Control tests required for cell culture rabies vaccine at various stages of the production process (ref.37)

1. Source materials

1.1. **Virus seed lot**: see summary of control tests for poliomyelitis vaccine.

1.2. **Cell bank** (continuous cell lines, VERO cells : see summary of control tests for poliomyelitis vaccine.

1.3. **Bovine serum** : see summary of control tests for poliomyelitis vaccine.

1.4. **Trypsin** : see summary of control tests for poliomyelitis vaccine.

2. **Cell culture** (production) : see summary of control tests for poliomyelitis vaccine.

3. **Virus culture** (before filtration) :
- tests for extraneous agents in tissue cultures (4 weeks).
- tests for bacterial and mycotic sterility
- virus titre (mice) (2 weeks)
- antigen content (ELISA)

4. **Virus harvest** (after concentration and purification) :
- virus titre (mice) (2 weeks)
- antigen content by *in vitro* test (ELISA or other suitable method)
- tests for bacterial and mycotic sterility

5. Bulk vaccine (fluid final bulk) :
- tests for residual live virus in tissue cultures and animals (mice) (7 weeks)
- antigen content by in vitro test (ELISA or other suitable method)
- bacterial and mycotic sterility
- protein nitrogen content (Kjeldahl)
- bovine serum protein (counter-current electrophoresis)
- test for residual cellular DNA and RNA

5. Lyophilized vaccine :
- identity test (potency test may serve as identity test)
- bacterial and mycotic sterility
- innocuity (in mice and guinea pigs) (10 days)
- potency test in vivo (NIH test) (ref. 39) (4 weeks)
- stability test (4 weeks at 37 °C, NIH test) (8 weeks)
- pyrogenicity test (in rabbits or by Limulus amoebocyte lysate test)
- residual moisture
- BPL-content

Control tests required for measles vaccine (live) prepared in primary chick embryo cells (ref.35) at various stages of the production process

1. Source materials

1.1. Virus seed lot: see summary of control tests for poliomyelitis vaccine.

1.2. Chicken flock (eggs for cell culture production): chickens from which embryonated eggs, used for cell culture production, are derived should be kept in a closed colony regularly monitored (ref.40) for presence of the following extraneous agents: avain leucosis viruses, reticulo-endotheliosis virus, influenza A virus, reoviruses, adenoviruses and the agents causing infectious bronchitis, infectious bursal disease, Marek disease, Newcastle disease, avian encephalomyelitis, avian laryngotracheitis and avian pox-diphtheria; Mycobacterium tuberculosis avium, Mycoplasma gallisepticum, Mycoplasma synoviae, Salmonella pullorum/gallinarum, Hemophilus sp. and other agents spontaneously infecting chickens.
Monitoring is mostly done by serological techniques and should preferably be performed by a veterinary institute specialized in avian diseases.

1.3. Bovine serum : see summary of control tests for poliomyelitis vaccine.

1.4. Trypsin : see summary of control tests for poliomyelitis vaccine.

2. Cell culture (production) :
- bacterial and mycotic sterility
- mycoplasma
- control cell cultures of the production culture shall be tested for the presence of hemadsorbing and cytopathogenic agents (2 weeks or as long as production culture runs)
- supernatant fluids of the control cell cultures shall be tested for extraneous agents in human, simian and chick embryo cells and suckling mice and for avian leukosis virus (5 weeks)

3. Virus harvest

3.1. Virus harvest (single) :
- bacterial and mycotic sterility
- virus content

3.2. Virus harvest (pooled, prior to clarification) :
- extraneous agents in human, simian and chick embryo cells, adult mice, suckling mice, embryonated chicken eggs (4 weeks)
- avian leucosis virus (4 weeks)
- bacterial and mycotic sterility
- mycoplasma
- Mycobacterium tuberculosis

4. Final bulk : bacterial and mycotic sterility

5. Lyophilized vaccine :
- identity (ref.35)
- potency (virus titre) (ref.35) (2 weeks)
- stability (1 week 37°C) (ref.35) (ref.40, Annex 6, Addendum 1981) (3 weeks)
- bacterial and mycotic sterility
- innocuity (in mice and guinea pigs) (10 days)
- animal (bovine) serum proteins (counter-current electrophoresis)
- residual moisture

2.3. Buildings and Services

2.3.1. Construction and construction materials

As given in Chapter 4.

2.3.2. Function, basic design and equipment

A comprehensive quality control department covering bacterial as well as viral vaccines requires different laboratory facilities related to the different types of testing needed and a facility for testing materials in animals. The quality control facilities described are given in square meter for the actual working areas. For corridors and services, another 20 to 30% surface area should be added. The estimates are based on a requirement for control testing of approximately one million doses of diphtheria-, pertussis-, tetanus-, measles (live)- and trivalent inactivated poliomyelitis vaccine and 100 thousand doses of tissue culture rabies vaccine. It should be realized, however, that the control capacity, as related to number of doses, is grossly influenced by other factors, e.g. batch size, and scheduling of production.

2.3.2.1. Chemical, biochemical and immunochemical laboratory facility (90 m^2)

Function: This laboratory has to effect tests for identity and quality of starting materials, including media and water, and has to determine the concentration of additives such as adjuvants, stabilizing agents and preservatives. The facility has also to test for the presence of residual inactivating agents such as formalin and beta-propiolactone and to determine protein nitrogen concentrations in final products. In addition this laboratory may perform tests for the presence of residual animal serum proteins in viral vaccines, tests for identity of cell substrates by isoenzyme analysis, and tests for antigen content of vaccines by immunochemical methods such as immunodiffusion or ELISA. Also tests for the presence of pyrogens by the Limulus amoebocyte lysate test, flocculation tests on bacterial vaccines, pH testing and testing for residual moisture of lyophilized vaccines may be effected in this laboratory. These functions require about 90 m^2 in a single large laboratory.

Vaccines produced on VERO cells have to be tested for residual cellular DNA and RNA will have to be performed. For this type of testing at present sensitive techniques, using molecular hybridization with radioactive probes, are applied. Additional facilities for this work with radioactive materials will require an estimated 50-60 m^2 as a separate laboratory.

Equipment: Essential services include electricity, gas, cold and hot water, washing up facilities, with a supply of good quality water (usually demineralized) for rinsing and a supply of high quality water (demineralized and distilled) for making solutions for analytical work. Vacuum and compressed air, if not available as central service, may be supplied by a laboratory vacuum/compression pump.

The laboratory should be equipped for routine chemical and biochemical work with sufficient bench space, cupboards for glassware, chemicals and apparatus and should be provided with a fume cupboard with a good exhaust fan.

Apparatus should include pH meters of sufficient precision, equipment for protein nitrogen determination by Kjeldahl analysis, spectrophotometers (general purpose and preferably also including a scanner for ELISA). Other equipment required includes balances (analytical and technical), centrifuge (general purpose with multiple loads, with refrigeration) electrophoresis equipment, waterbaths, refrigerator, freezer, blenders (homogenizers) shakers, filters.

When testing for residual DNA and RNA is required, there should be provisions for storage, handling and disposal of radioactive materials, a provision for dark room work and additional apparatus as a scintillation counter, apparatus for monitoring radioactivity, a high-speed centrifuge, spectrophotometer, UV lighting photography equipment, lighting plus screen, electrophoresis equipment with power supply, dot-blot apparatus, shaker (controlled environment incubator shaker), sealing equipment, refrigerator, deep-freezers (-20°C, -70°C) whirl-mixer, balances and other laboratory equipment.

2.3.2.2. Laboratory facility for microbiological testing (175 m^2)

Function:

a. Testing of source materials, vaccines (bacterial and viral) and intermediate products for bacterial and mycotic sterility.

b. Testing of viral vaccines and source materials for mycoplasma. Required is area, separate from (a) and (c), divided into two parts, i.e. one part for the actual testing and the other part for work with live reference organisms.

c. Testing of BCG for potency (culturable particles), stability and identity.

d. Monitoring of (microbiological) environmental quality, characterization of isolated organisms, antimicrobial preservative effectiveness testing.

e. Storage of retained reference samples (this being a responsibility of the quality control department). Storage facility may either be cold room or special room with separate refrigerators and deep-freezers. Combination with storage of media is acceptable.

Equipment: Besides essential services as electricity, water and gas a filtered air supply is very recommendable but not absolutely necessary if the laboratory is supplied with laminar flow safety cabinets. In addition to laminar flow cabinets, including "Biohazard" safety cabinets, the equipment should include laboratory incubators (22°C, 30°C, 37°C) refrigerators, deep-freezers (-20°C, -70°C), microscopes (including one suitable for fluorescent microscopy for mycoplasma testing), equipment for anaerobic testing and centrifuges. A steam supply or autoclave is not required provided infected materials and equipment can be transported to an autoclave in the central service area.

2.3.2.3. Laboratory facility for virological control of virus vaccines (175 m^2)

Function:

a. Testing of control cells of production cultures and source materials for adventitious agents in tissue cultures and testing of inactivated virus vaccines for residual live virus in tissue cultures. To prevent cross-contamination this area should be well-separated from areas where live virus suspensions are handled i.e. b and c.

b. Testing of (live) virus suspensions, including seed lots, for adventitious viruses in tissue cultures (all virus vaccines) and in embryonated eggs (measles virus).

c. Testing of live (measles) vaccine for identity, potency and stability, determination of virus inactivation rate (poliomyelitis vaccine) identity testing of polio vaccine virus, testing of sensitivity of cell cultures, used in control work, for poliovirus and other viruses, preparation of vaccine dilutions and challenge virus suspensions for tests in animals, serological work related to potency testing of vaccines in animals and for measuring antibody responses in man after vaccination, if required. Also serological testing of animals for (animal) virus infections and initial characterization of isolated adventitious agents may sometimes be required.

d. Production of cell cultures for control from other areas testing of viral vaccines. This area should be well-separated and should include facility for freezing and storage of cell cultures in liquid nitrogen.

e. Storage of retained reference samples related to virus vaccine testing at refrigerator temperature and at -70°C. This function may be shared with similar function for bacterial vaccines (see section 2.3.2.2.).

Equipment: In addition to essential services as electricity, gas and water, there should be sufficient bench space and cupboards for storing materials. A filtered air supply is recommendable. Equipment should include laminar flow safety cabinets (including

"Biohazard"), refrigerators, deep-freezers (-20°C, -70°C), liquid nitrogen containers for storage of cell cultures and for transport of nitrogen, freezing equipment for cell cultures, centrifuges, laboratory incubators, egg incubator, waterbaths (different temperatures), microscopes (including fluorescence) equipment for microtiter work, laboratory vacuum/compressed air pumps and other small laboratory equipment. An autoclave is required when infected materials and equipment cannot be transported to an autoclave in the central service area. Facilities for washing up of glassware from tissue culture and production of tissue culture media are not included here as it is assumed that these are provided by the central services. There should be some containment facilities in areas where work is performed with virulent virus strains.

2.3.2.4. Facilities for safety and potency testing of bacterial and viral vaccines in animals (310 m^2)

a. Laboratory facility

Function: Most of the quality control function related to bacterial vaccines is performed in the experimental animal house. However some laboratory facilities are needed for preparation of vaccine dilutions, bacterial challenge suspensions for potency testing and other biological work.

Equipment: Refrigerator, deep-freezer, laboratory incubators. In addition to essential services as electricity, gas and water, laboratory bench space, a refrigerator, deep-freezer and laboratory incubator are required. Storage space for retained reference samples may be shared with the facility mentioned in section 2.3.2.2.

b. Animal facilities

Function:

a. Testing of all vaccines (final product) and reconstitution fluids for innocuity (abnormal toxicity) in mice and guinea pigs.

b. Testing of bacterial vaccines for specific toxicity in guinea pigs (diphtheria and tetanus) and mice (pertussis).
c. Testing of inactivated bacterial and viral vaccines for potency in mice (pertussis, rabies, tetanus), guinea pigs (diphtheria) and rats or other suitable animals (polio).
d. Testing of BCG for virulent mycobacteria (guinea pigs) and for skin reactivity (guinea pigs).
e. Testing of suspensions of (live) vaccine virus suspensions for adventitious agents in suckling and/or adult mice.
f. Testing of rabies virus suspension for virus titre.
g. Testing of rabies vaccine for residual live virus.
h. Testing of cell substrates (subcultured cells and cell lines) for tumorigenicity in immunosuppressed animals.
i. Pyrogen testing in rabbits.

Building design: The animal control facility should be separate from the animal breeding facility, if required, and should allow for separation of species, to help in control of infectious diseases. In this respect multiple small rooms that can be easily cleaned and desinfected after each experiment, are preferable to larger rooms. There should be some containment facilities for work with infectious agents such as live (virulent) poliomyelitis virus and rabies virus.

As it is well-known that such factors as physical environment, food and infections of various types may profoundly affect biological functions, including the immune response of animals, a facility for animal testing should ideally have a controlled environment, i.e. a system permitting individual adjustment of temperature, humidity, light and ventilation/air pressure, and should be fully protected against unwanted introduction of infectious agents by for example wild rodents, insects, and other means. It should be kept in mind, however, that even in a well-designed facility only good and consistent test results can be obtained, provided genetically well-characterized, healthy animals of high quality microbiological status can be obtained and provided high quality food, water and other necessities are available. More detailed information on the design of animal houses, animal production, care and use is given elsewhere (ref. 12, 41, 42, 43, 44).

Provisions should include:

1. Rooms for mice
2. Rooms for guinea pigs
3. Room for rabbits
4. Rooms for rats or other small laboratory species
5. Inoculation room or, preferably, inoculation area within each animal room
6. Post-mortem and clinical diagnostic laboratory
7. Storage of feed and bedding
8. Cage washing and desinfection
9. Autoclave
10. Storage of clean cages and other equipment
11. Incinerator for burning animal waste and dead animals
12. Quarantine rooms isolated from rest of facility if animals are brought into the facility from outside sources
13. Toilet facilities, showers, changing area
14. Office for facility staff

Rooms for quarantine, holding and use of primates are not included in this facility as for inactivated poliomyelitis vaccine there is no requirement for primates unlike live (oral) poliovaccine where neurovirulence testing in monkeys is required for each monovalent pool.

Equipment: Essential services include electricity, hot and cold water. In addition to cages, racks, feeders, water bottles, a refrigerator for storing dead animals, equipment should also include apparatus for narcosis of animals and equipment for gross pathological examination. Special attention should be paid to equipment for cleaning and desinfection of rooms and cages.

To the working area of 750 m^2 for laboratory and animal testing of vaccines, mentioned so far, about 200 m^2 has to be added for general purpose such as administrative work, technical facilities etc.

Figure 2.1. shows a model for the flow of protocols and test samples to the different facilities for quality control.

2.4. Space, budget for equipment and personnel required for quality control of bacterial and viral vaccines

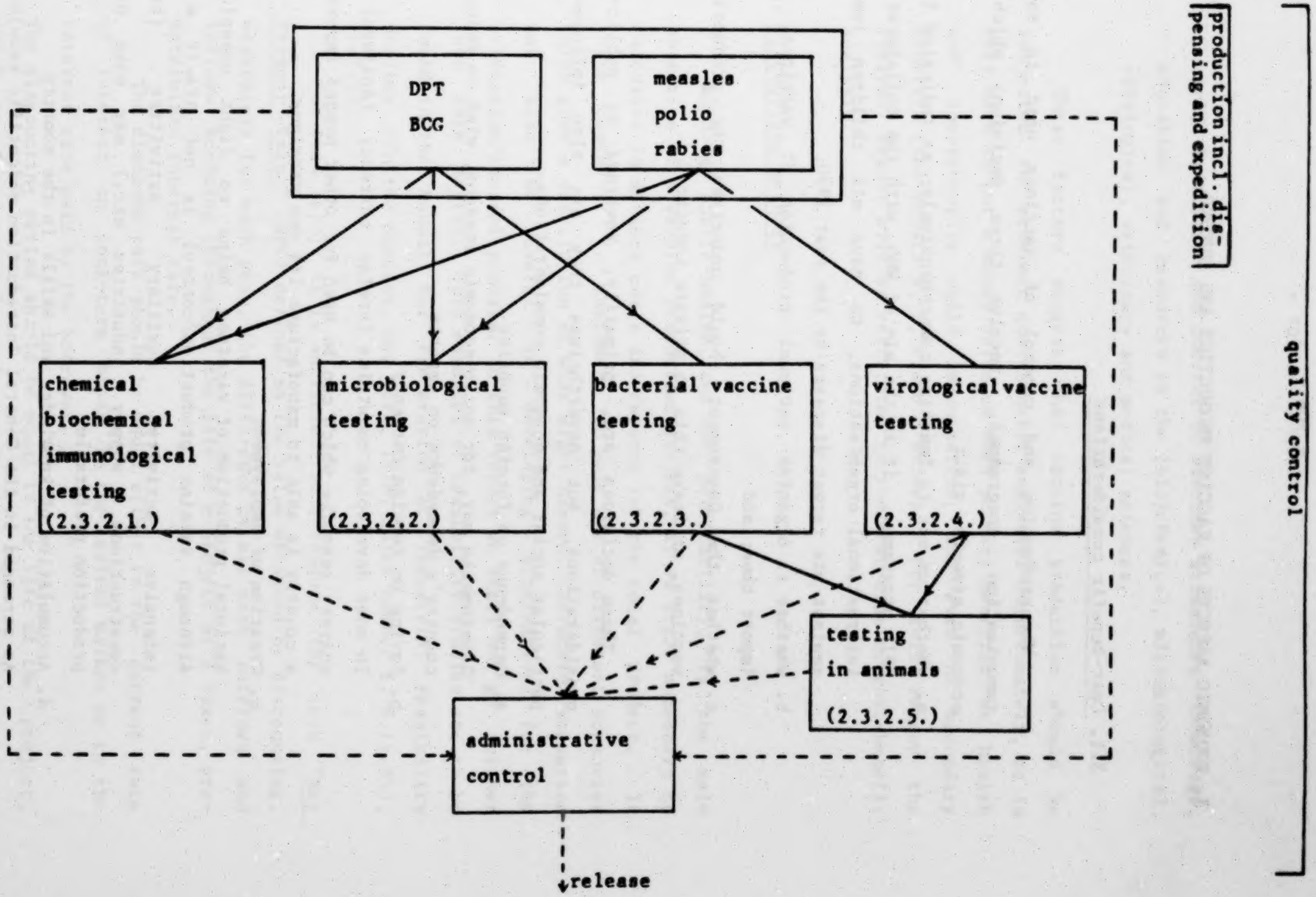
Space, budget for equipment and personnel required for quality control of bacterial and viral vaccines, are summarized in Table 2.1.

Table 2.1

Space, budget for equipment and personnel required for quality control of bacterial and viral vaccines

	Population of	
	10 million	100 million
Space	750 m^2	935 m^2
Budget for equipment in Dutch guilders	2.0 million	2.4 million
Personnel:		
head or director of quality control	1	1
(bio)chemist	1	1
microbiologist	1	2
virologist	1	1
veterinarian	1 (50%)	1 (50%)
senior technicians	6	8
technicians	9	13
other personnel	5	8

Figure 2.1. Simplified model for the flow of test samples (—>) and protocols (-->) for quality control



3. ECONOMIC ASPECTS OF VACCINE PRODUCTION AND CONTROL

3.1. Cost-benefit considerations

The production and control of vaccines used in national immunization programmes, involve three decisions which have economic aspects, viz.:

a. whether or not to immunize comprehensively; as indicated in its EPI programme, it is the aim of WHO, with the collaboration of international organizations, to have all children immunized against six target diseases by the year 1990;

b. whether to organize national production of vaccines, or to import them; and

c. whether the Government should undertake the production of vaccines, or leave this to private industry.

These decisions are, primarily, governed by public-health considerations, but nevertheless they are also influenced by particular social and economic considerations.

a. Dependence on foreign supplies

Governments may, for socio-economic reasons, wish to reduce the country's dependence on imports.

b. Saving on foreign currency

In most developing countries foreign currency (dollars) is a scarce resource which can be used for other needed imports when a country is able to manufacture its own vaccines.

c. Creation of employment

National production of vaccines helps to fight unemployment. Although vaccine production/control is not itself a labour intensive activity, auxiliary activities (building constructions, supply industries etc.) may make national production preferable.

d. Accumulation of knowledge and skills in the country

As stressed in the General Introduction scientific knowledge is very much concentrated in certain centres in the developed world. Production and control of vaccines stimulates knowledge, education and resources in the (bio)chemical, microbiological, virological, veterinary and medical sciences.

These factors suggest that vaccine production should be subjected to economic cost-benefit analysis. Unfortunately, it is very hard to put a money value on such primary benefits as health and comprehensive child immunization, and even on secondary benefits (see points a, b, c and d above). For this reason the present report will be restricted to the cost side of cost-benefit analysis.

3.2. Discounting methods

Although public health, and not profitability, is the main objective of a vaccine production project, it is still necessary to calculate in advance costs and returns for the total project. In these cases it is customary to make use of different scenarios which give a description of what might happen. Economic scenarios can also be useful in developing alternatives for the production/control process. In this report we shall not go further into this matter. Good examples of feasibility studies are to be found in the Manual for Preparation of Industrial Feasibility Studies (United Nations, New York, 1978, sales number E.78.11.B5), (ref.2).

For this report it is sufficient to introduce the term "Net Present Value" (NPV) defined as the value obtained by discounting, separately for each year, the difference of all cash outflows and inflows accruing throughout the life of a project at a fixed, predetermined interest rate.

The discount rate should be equal either to the current rate of interest on long-term bonds in the capital market or to the interest rate paid by the borrower.

The discounting period should be equal to the life of the project, which in the case of vaccine production and control is taken to last 25 to 40 years. (It is evident, that as a result of rapidly increasing scientific knowledge, the techniques can change in the meantime).

3.3. Distribution network; economic of scale

Of greatest importance for an immunization project and for vaccine production is the distribution network; the supply routes for raw materials, energy, laboratory animals are also important.

From the viewpoint of distribution of vaccines it seems preferable to have several production and control centres spread over the country. But the distribution of raw materials and laboratory animals argues for a single production centre.

In most cases economic arguments will tip the balance. As becomes evident from the cost calculations in this report (appendices 1 to 12) there is already a great difference in costs of production/control of vaccines for a population of 10,000,000 or 100,000,000 people. Therefore only when extremely large quantities are needed, will it be economical to create more than one production/control centre.

As situations are quite different from country to country it is impossible to present in this report a solution that would be applicable to all of them. In most cases, however, a single production/control centre will be the most efective option.

3.4. Costs of production/control/filling and packaging

Several studies on cost-benefit analysis of vaccination have been published in recent years. They will not be surveyed here, but for those interested documents (ref. 45-59) are available.

The present documentation covers procedures and operations from the moment when a Government (or private organization), after cost-benefit studies and a critical assessment of the situation in the country, decides to undertake the production of one or several vaccines.

Costs are different for each vaccine and they vary from country to country (see also ref.2).

One can, however, show in a general model the various cost factors connected with vaccine production. This general model, presented in appendix 13, attempts to take into account specific situations which may exist elsewhere in the world. The RIVM model has been worked out in Appendices 1 to 12. The odd-numbered appendices for 10,000,000 persons, the even ones for 100,000,000 persons.

Appendix 1 and 2: central kitchen
Appendix 3 and 4: production unit of bacterial vaccines
Appendix 5 and 6: production unit of inactivated virus vaccines
Appendix 7 and 8: production unit of live virus vaccines
Appendix 9 and 10: filling and packaging
Appendix 11 and 12: quality control unit

Below the factors used to prepare the models are given.

3.4.1. Capital costs

Capital costs are defined as the costs of investment in buildings, machinery, equipment and expertise, which are subject to depreciation over prolonged periods of time.

3.4.1.1. Buildings

The cost factor reflects the cost of construction, or acquisition, of buildings (incl. the land, installations and air conditioning), used for production and related operations (cultivation, harvesting, purification, inactivation, mixing, filling, control). It consists essentially of annual depreciation and interest. Customary depreciation periods are 50 years for buildings, 15 years for installations, none for the land.

At the RIVM cost of similar laboratory buildings (incl. energy) are Dfl. 600.- per m^2.

3.4.1.2. Machinery and equipment

This heading refers to the acquisition, delivery and installation of various types of machinery and equipment necessary for completion of the total production and control process, and not covered under item "Materials" (see chapter 1 and 2). As in the case of buildings, the cost is made up of annual depreciation (10%) and interest (5%).

3.4.1.3. Expertise

Buildings and machinery together are not sufficient to start production of vaccines. Expertise in the choice and development of vaccine production/control procedures is also a capital cost. However, such a cost factor is usually difficult to appraise.

The costs of the purchase of a strain are rather stable, but those of the subsequent development work can range enormously. In some cases the transfer of technology and expertise is placed at a country's disposal at no cost.

In the appendices the RIVM costs of expertise are used as those ascribed to "Research and Development".

3.4.2. Running costs

Running costs are defined as yearly recurring expenditures (manpower, material, etc.), necessary to ensure continuation of the production process.

3.4.2.1. Manpower

Salaries and allowances comprise the cost of manpower. For a first subdivision of salary costs one may distinguish between university level staff, senior technicians and technicians. (In the RIVM model respectively ƒ 120,000.-, ƒ 65,000.- and ƒ 50,000.-) Manpower costs vary widely. In extreme situations (countries with a high unemployment rate) they could, from a macro-economic viewpoint, approach zero.

3.4.2.2. Materials

These include essentially media, basic chemicals, vials, seals, packaging materials, animals, etc. For most materials, the expenditures are proportional to production volume.

3.4.2.3. Supporting staff

Salaries and allowances paid to the staff of supporting services (technical department, administration, laboratory-animal facilities, glassware cleaning etc.) make up this cost factor.
It is advisable to distinguish, where possible, between services rendered which can be charged directly to a particular vaccine (direct support) and others (overhead). Technical staff represent an example of the first category, administration of the second.
If the actual amount of overhead costs is unknown, they may be put at 30% of all labour costs based on experience at the RIVM.

3.4.2.4. Energy consumption

This item includes cost of electricity and gas. At the RIVM energy costs are for practical reasons added (as a percentage) to the yearly costs for buildings (see 3.4.1.1), but in most cases it will be more convenient to list them separately.

3.4.2.5. Maintenance and repair

For a smoothly running vaccine production good maintenance and repair staff are essential. In the research and development phase, these costs must be allocated. Once production has been organized, they are incorporated into the Supporting Staff, as direct support if possible, otherwise as overhead expense.

3.4.2.6. Research and Development (R&D)

While "Expertise" is viewed as a form of capital, R&D is considered to be a part of running costs.

If at the outset it is possible to establish the actual size of R&D expenditures, they should be accounted us with a percentage of the cost of production. In the RIVM model the annual costs of R&D and of expertise are left out of account. A very rough estimation of these costs is 100% of all the other running costs.

3.4.3. Total costs for the separate vaccines and costs per vaccinated child

In table 3.1 the total costs are given for the vaccines DPT, BCG, polio, rabies and measles. These figures give a rough estimation of the costs per dose of each vaccine.

As the figures in appendix 1 to 12 are a combination of the existing RIVM costs and the design described in chapter 4, they are only an estimate.

With the same restriction the indication of the vaccination costs per child for an immunization schedule of 3 doses DPT and polio and one dose of BCG and measles are ƒ 26.40 and ƒ 5.50 based on the production for a population of 10,000,000 and 100,000,000 respectively.

Table 3.1

Population of 10,000,000

	DPT 1,000,000 doses	BCG 400,000 doses	Polio 1,000,000 doses	Rabies 100,000 doses	Measles 400,000 doses
Production	1,022,400	255,600	1,316,700	564,300	1,181,000
Control	821,000	273,600	1,094,700	547,300	547,400
Kitchen	302,000	100,700	402,700	201,300	201,300
Filling	619,000	325,000	619,000	80,000	325,000
Total	2,764,400	954,900	3,433,100	1,392,900	2,254,700
per dose	ƒ 2.76	ƒ 2.39	ƒ 3.43	ƒ 13.93	ƒ 5.64

Population of 100,000,000

	DPT 1,000,000 doses	BCG 400,000 doses	Polio 1,000,000 doses	Rabies 100,000 doses	Measles 400,000 doses
Production	1,356,400	339,100	4,854,500	2,080,500	2,540,500
Control	1,329,000	443,000	1,772,000	886,000	886,000
Kitchen	466,600	155,500	622,200	311,100	311,100
Filling	1,540,700	721,000	1,540,800	180,000	721,000
Total	4,692,700	1,658,600	8,789,500	3,457,600	4,458,600
per dose	ƒ 0.47	ƒ 0.41	ƒ 0.88	ƒ 3.46	ƒ 1.11

RIVM MODEL

Appendix 1

Central Kitchen
Population of 10,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 360 x f.600.- = f.216,000.-

1.2 Machinery and equipment

depreciation 10% x purchase value	= f.150,000.-
interest 5 x % x balance-sheet value	= f. 75,000.-
total costs	f.225,000.-

2. RUNNING COSTS

2.1 Manpower

academics	1 years x f.120,000.-	= f.120,000.-
senior technicians	2 years x f. 65,000.-	= f.130,000.-
technicians	2 years x f. 50,000.-	= f.100,000.-
total costs		f.350,000.-

2.2 Materials

f.

2.3 Supporting staff

direct support	6 years x f. 40,000.-	= f.240,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.177,000.-
	total costs	f.417,000.-

Total f.1,208,000.-

RIVM MODEL **Appendix 2**

Central Kitchen
Population of 100,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 520 x f.600.- = f.312,000.-

1.2 Machinery and equipment

depreciation 10% x purchase value	= f.200,000.-
interest 5 x % x balance-sheet value	= f.100,000.-
total costs	f.300,000.-

2. RUNNING COSTS

2.1 Manpower

academics	1 years x f.120,000.-	= f.120,000.-
senior technicians	3 years x f. 65,000.-	= f.195,000.-
technicians	5 years x f. 50,000.-	= f.250,000.-
total costs		f.565,000.-

2.2 Materials

f.

2.3 Supporting staff

direct support	10 years x f. 40,000.-	= f.400,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.289,500.-
total costs		f.689,500.-

Total f.1,866,500.-

RIVM MODEL **Appendix 3**

Production of bacterial vaccines
Population of 10,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 360 x f.600.- = f.216,000.-

1.2 Machinery and equipment

depreciation 10% x purchase value = f.150,000.-
interest 5 x % x balance-sheet value = f. 75,000.-
total costs f.225,000.-

2. RUNNING COSTS

2.1 Manpower

academics 2 years x f.120,000.- = f.240,000.-
senior technicians 2 years x f. 65,000.- = f.130,000.-
technicians 3 years x f. 50,000.- = f.150,000.-
total costs f.520,000.-

2.2 Materials f. 5,000.-

2.3 Supporting staff

direct support 3 years x f. 40,000.- = f.120,000.-
overhead 30% x labour costs (2.1 + 2.3) = f.192,000.-
total costs f.312,000.-

Total f.1,278,000.-

Production of bacterial vaccines
Population of 100,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 520 x f.600.-	= f.312,000.-

1.2 Machinery and equipment

depreciation 10% x purchase value	= f.200,000.-
interest 5 x % x balance-sheet value	= f.100,000.-
total costs	f.300,000.-

2. RUNNING COSTS

2.1 Manpower

academics	2 years x f.120,000.-	= f.240,000.-
senior technicians	3 years x f. 65,000.-	= f.195,000.-
technicians	4 years x f. 50,000.-	= f.200,000.-
total costs		f.635,000.-

2.2 Materials

f. 50,000.-

2.3 Supporting staff

direct support	4 years x f. 40,000.-	= f.160,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.238,500.-
	total costs	f.398,500.-

Total	f.1,695,500.-

RIVM MODEL **Appendix 5**

Production of inactivated virus vaccines
Population of 10,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 415 x f.600.- f.249,000.-

1.2 Machinery and equipment

depreciation 10% x purchase value	= f.200,000.-
interest 5 x % x balance-sheet value	= f.100,000.-
total costs	f.300,000.-

2. RUNNING COSTS

2.1 Manpower

academics	2 years x f.120,000.-	= f.240,000.-
senior technicians	2 years x f. 65,000.-	= f.130,000.-
technicians	3 years x f. 50,000.-	= f.150,000.-
total costs		f.520,000.-

2.2 Materials

f.500,000.-

2.3 Supporting staff

direct support	3 years x f. 40,000.-	= f.120,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.192,000.-
total costs		f.312,000.-

Total f.1,881,000.-

RIVM MODEL **Appendix 6**

Production of inactivated virus vaccines
Population of 100,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 620 x f.600.- = f.372,000.-

1.2 Machinery and equipment

depreciation 10% x purchase value	= f.275,000.-
interest 5 x % x balance-sheet value	= f.137,500.-
total costs	f.412,500.-

2. RUNNING COSTS

2.1 Manpower

academics	2 years x f.120,000.-	= f.240,000.-
senior technicians	3 years x f. 65,000.-	= f.195,000.-
technicians	5 years x f. 50,000.-	= f.250,000.-
total costs		f.685,000.-

2.2 Materials

f.5,000,000.-

2.3 Supporting staff

direct support	5 years x f. 40,000.-	= f.200,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.265,500.-
	total costs	f.465,500.-

Total f.6,935,000.-

RIVM MODEL Appendix 7

Production of live virus vaccines
Population of 10,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 360 x f.600.- = f.216,000.-

1.2 Machinery and equipment

depreciation 10% x purchase value	= f.100,000.-
interest 5 x % x balance-sheet value	= f. 50,000.-
total costs	f.150,000.-

2. RUNNING COSTS

2.1 Manpower

academics	2 years x f.120,000.-	= f.240,000.-
senior technicians	2 years x f. 65,000.-	= f.130,000.-
technicians	2 years x f. 50,000.-	= f.100,000.-
total costs		f.470,000.-

2.2 Materials

f.100,000.-

2.3 Supporting staff

direct support		2 years x f. 40,000.-	= f. 80,000.-
overhead	30% x labour costs (2.1 + 2.3)		= f.165,000.-
	total costs		f.245,000.-

Total f.1,181,000.-

RIVM MODEL **Appendix 8**

Production of live virus vaccines
Population of 100,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 470 x f.600.- = f.282,000.-

1.2 Machinery and equipment

depreciation	10% x purchase value	= f.150,000.-
interest	5 x % x balance-sheet value	= f. 75,000.-
total costs		f.225,000.-

2. RUNNING COSTS

2.1 Manpower

academics	2 years x f.120,000.-	= f.240,000.-
senior technicians	3 years x f. 65,000.-	= f.195,000.-
technicians	4 years x f. 50,000.-	= f.200,000.-
total costs		f.635,000.-

2.2 Materials f.1,000,000.-

2.3 Supporting staff

direct support	4 years x f. 40,000.-	= f.160,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.238,500.-
total costs		f.398,500.-

Total	f.2,540,500.-

RIVM MODEL

Appendix 9

Filling and Packaging
Population of 10,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 625 x f.600.- = f.375,000.-

1.2 Machinery and equipment

depreciation	10% x purchase value	= f.300,000.-
interest	5 x % x balance-sheet value	= f.150,000.-
total costs		f.450,000.-

2. RUNNING COSTS

2.1 Manpower

academics	2 years x f.120,000.-	= f.240,000.-
senior technicians	2 years x f. 65,000.-	= f.130,000.-
technicians	2 years x f. 50,000.-	= f.100,000.-
total costs		f.470,000.-

2.2 Materials

f.220,000.-

2.3 Supporting staff

direct support	6 years x f. 40,000.-	= f.240,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.213,000.-
	total costs	f.453,000.-

Total	f.1,968,000.-

Filling and packaging
Population of 100,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 930 x f.600.- = f.558,000.-

1.2 Machinery and equipment

depreciation	10% x purchase value	= f.400,000.-
interest	5 x % x balance-sheet value	= f.200,000.-
total costs		f.600,000.-

2. RUNNING COSTS

2.1 Manpower

academics	2 years x f.120,000.-	= f.240,000.-
senior technicians	3 years x f. 65,000.-	= f.195,000.-
technicians	4 years x f. 50,000.-	= f.200,000.-
total costs		f.635,000.-

2.2 Materials f.2,200,000.-

2.3 Supporting staff

direct support	10 years x f. 40,000.-	= f.400,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.310,500.-
total costs		f.710,500.-

Total f.4,703,500.-

RIVM MODEL **Appendix 11**

Quality control
Population of 10,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per quare meter incl.energy)

yearly expenses (depreciation, interest): 750 x f.600.- = f.450,000.-

1.2 Machinery and equipment

depreciation 10% x purchase value	= f.200,000.-
interest 5 x % x balance-sheet value	= f.100,000.-
total costs	f.300,000.-

2. RUNNING COSTS

2.1 Manpower

academics	4,5 years x f.120,000.-	= f.540,000.-
senior technicians	6 years x f. 65,000.-	= f.390,000.-
technicians	9 years x f. 50,000.-	= f.450,000.-
total costs		f.1.380,000.-

2.2 Materials f.480,000.-

2.3 Supporting staff

direct support	5 years x f. 40,000.-	= f.200,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.474,000.-
total costs		f.674,000.-

Total f.3,284,000.-

Appendix 12

Quality control
Population of 100,000,000 people

1. CAPITAL COSTS

1.1 Buildings (costs per square meter incl.energy)

yearly expenses (depreciation, interest): 935 x f.600.- = f.561,000.-

1.2 Machinery and equipment

depreciation	10% x purchase value	= f.240,000.-
interest	5 x % x balance-sheet value	= f.120,000.-
total costs		f.360,000.-

2. RUNNING COSTS

2.1 Manpower

academics	5,5 years x f.120,000.-	= f.660,000.-
senior technicians	8 years x f. 65,000.-	= f.520,000.-
technicians	13 years x f. 50,000.-	= f.650,000.-
total costs		f.1,830,000.-

2.2 Materials

f.1,600,000.-

2.3 Supporting staff

direct support	8 years x f. 40,000.-	= f.320,000.-
overhead	30% x labour costs (2.1 + 2.3)	= f.645,000.-
	total costs	f.965,000.-

Total	f.5,316,000.-

GENERAL MODEL

Appendix 13

1. CAPITAL COSTS:

1.1 Buildings (excl.energy)

- land : interest: x % balance-sheet value = $ ....
- building: depreciation: 2% x purchase value = $ ....
interest: x % balance-sheet value = $ ....
- installations : depreciation: 7% x purchase value = $ ....
interest: x % balance-sheet value = $ ....
total costs: $ ......

1.2 Machinery and equipment

depreciation 10% x purchase value = $ ....
interest: x % balance-sheet value = $ ....
total costs: $ ......

1.3 Expertise

depreciation 10% x purchase value = $ ....
interest: x % balance-sheet value = $ ....
total costs: $ ......

2. RUNNING COSTS:

2.1 Manpower

academics ..days x $ .... = $ ....
senior technicians ..days x $ .... = $ ....
technicians ..days x $ .... = $ ....
total costs $ ......

2.2 Materials $ ......

2.3 Supporting staff

direct support technical ..days x $ .... = $ ....
cleaning ..days x $ .... = $ ....
etc. ..days x $ .... = $ ....
overhead 30% x labour costs (2.1) = $ ....
total costs $ ......

2.4 Energy consumption $ ......

2.5 Maintenance and repairs $ ......

2.6 Research and development $ ......

Total $ ......

4. LAYOUT OF TECHNICAL FACILITIES OF PRODUCTION OF VACCINES

4.1 Architectural and technical facilities

4.1.1 Introduction

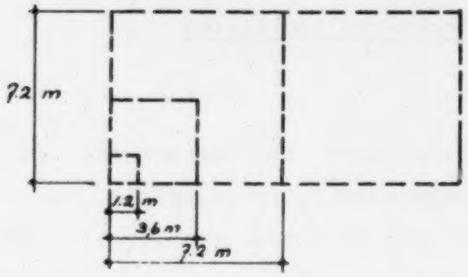
This report deals with the structural and infrastructural provisions that are necessary, in principle, for a building complex in which sufficient vaccine can be prepared for the 6 EPI vaccines and rabies vaccine for a population of about 100 million.

At the same time a global building complex has been designed in which similar production can be carried out, with an outline of the appropriate technical installations. The starting point of the design is vaccine production processes on a time sharing basis, through which the number of production units can be limited to the following three:

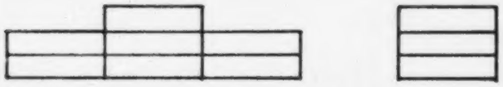
- in unit 1: tetanus, diphtheria, BCG and pertussis
- in unit 2: polio, 1, 2, 3 as well as rabies
- in unit 3: measles

These units also utilize a central kitchen, one mixing - filling - freeze-drying section, and several shared functions, centralized as much as possible, as well as a central power plant.

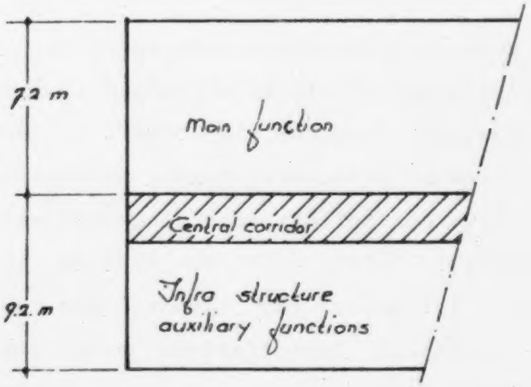
The vaccine production requires very specific preparation and processing methods, which should be organized in a manner allowing for the greatest possible control in order to achieve maximum consistency. The flow of personnel, goods, production material and waste must be strictly controlled according to written standing operating procedures. It will be obvious that the above requirements greatly influence the layout, the extent and the quality of the technical installations, the power supply, air conditioning, water treatment, waste and sewage-treatment, to name the most important. For these too we assume maximum centralization. The best techniques to apply depend strongly on local factors, for example climate and local infrastructure.



Construction model (fixed measurement)



Join and stack buildings elements



Structural design for a function

Fig.-4.1-

4.1.2 The architectural programme (fig. -4.1-)

The architectural programme compromises the following main points.

4.1.2.1 Construction measurement

The choice of design construction measurement falls on 7.2 x 7.2 m, a measurement used in many international applications (multiple of 60 cm), which satisfies construction and technical design requirements. A fixed design measurement has been chosen to keep open the possibility of joining all requisite building sections together in several different ways to stack them so that they always fit together. Many materials are also based on this measurement system, in order to keep the costs of a similar standard construction within reasonable bounds.

4.1.2.2 The frame

The materials to be utilized for the frame will depend to a great extent on the design, multi- or single-storey, and the building materials present in the locality. In principal, a choice of several possible materials needing little or no maintenance can be made for the frame.

4.1.2.3 Prefab construction

Due to standard construction dimensions for all buildings, it is possible to consider if required, the importation of prefabricated elements. This in the case that raw materials are too hard to get or too expensive.

4.1.2.4 Construction

Here too, it is important which materials are present in the country concerned. However, it should be stated beforehand that the construction of the area is determined by the function in that area and in view of the required hygiene, stringent demands are placed on the construction. In addition, the completion should be executed flexibly so that it can still be adapted for future requirements and wishes. Naturally, it is an advantage to adopt materials requiring little maintenance. Also, the work to be carried out should be of a high quality, which places requirements on the contractor to be chosen.

4.1.2.5 Fittings (furniture, apparaturs and equipment)

Although the fittings are not yet relevant at this stage, but they should be easy to keep clean and require little maintenance.

4.1.2.6 Inter-site connections

The connecting paths on the site should be level, sufficiently wide and preferably covered.

4.1.3 The technical programme

The technical programme can be described schematically in the following main points.

4.1.3.1 Air conditioning (air class definition according to American Standards)

In view of the very exacting requirements for GMP involved in vaccine production, air conditioning in the whole building complex is required, with recycling where possible. In the production units and support for the filling unit, an air class of 100,000 is required for the whole building. Air class of 10,000 - 1,000 is required for the vaccine production and filling areas without recycling. All activities requiring an even greater purity (class 100) will be carried out in the laminar air flow cells intended for that purpose.

4.1.3.2 Water supplies

A distinction can be made between the treatment and central distribution network for water in the following categories.

- water intended for consumption : for domestic use (drinking water and sanitary facilities, civil service); municipal or treated spring water

- water intended for installations : for installations (humidifying), cooling, washing machines, laboratories and sterilizing equipment); in principal softened or demi-water

- water intended for preparation processes : central treatment and pipe network (reversed osmosis or distillation) for the production units and filling section

The quantities are dependent on the number of tap points and capacity of the different installations.

4.1.3.3 Sewage system

Here, a distinction is made between provisions for sewage water and the treatment of fixed sewage.

- sewage water :
  - domestic and rain water: discharged straight into drains
  - process sewage water: not infected: discharged straight into drains
    infected: (thermal) disinfection followed by discharge

- treatment of fixed sewage : here we proceed from an installation for incinerating domestic sewage and disinfected process sewage

4.1.3.4 Power supply

We assume that prive generation of energy by gas or diesel generators, with spare capacity to allow for periodical maintenance. An emergency supply is desirable for continuation of the production processes (electrical energy, ventilation). The total supply is determined by the expected maximum simultaneous load, in which peak loads should be avoided.

4.1.3.5 Electrical installations

Electrical energy is distributed from the power plant to the distributors for each building. For this there are fittings namely:

- power installations (rotary current) for drive units
- light fittings for :
    - lighting
    - sockets
    - small scale equipment
- regulating and signalling fittings
- communication fittings :
    - telephone
    - intercom
    - data transmission

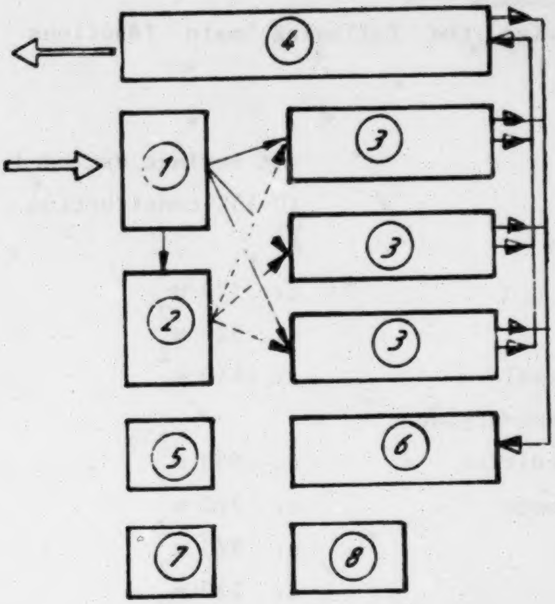
4.1.3.6 Provisions for gases

Central compressor installation for compressed air needed for:

- controlling air conditioning installations
- disconnecting couplings in work areas and garage
- controlling production equipment
- laboratory use

Located at the required work place, batteries of bottles for the different sorts of gas:

- work areas : oxygen/acetylene
- laboratories : propane/butane
- production units : CO2, O2 and N2
- mixing-filling station : CO2 and N2 where necessary



1 central storage
2 central kitchen
3 production-units
4 mixing-,filling-,freeze-drying,store and expedition
5 admin. and management
6 control laboratory facilities
7 civil services
8 power station and tech.-services.

schematic structure and flow patterns

Fig.- 4.2-

4.2 The structural design

4.2.1 Main functions and surface area (fig. -4.2-)

In the structural design the following main functions are linked:

	net surface excluded 10-15% construction
1. Central storage	c. 320 m^2
2. Central kitchen	c. 520 m^2
3. Production units (3 items)	c. 1610 m^2
4. Mixing-, filling-, freeze-drying station, store and expedition	c. 930 m^2
5. Administration, management	c. 220 m^2
6. Control laboratories	c. 920 m^2
7. Civil services	c. 220 m^2
8. Power plant and technical services	c. 620 m^2
TOTAL	c. 5400 m^2

The surface areas include c. 30% space for circulation. The animal breeding house is not included in this (surface area approx. 400 m^2 will be required).

4.2.2 The 'flow' pattern

Within the main structure the following flows can be identified:

- flow of personnel
- flow of goods (incoming, outgoing, and internal transport)
- flow of effluent

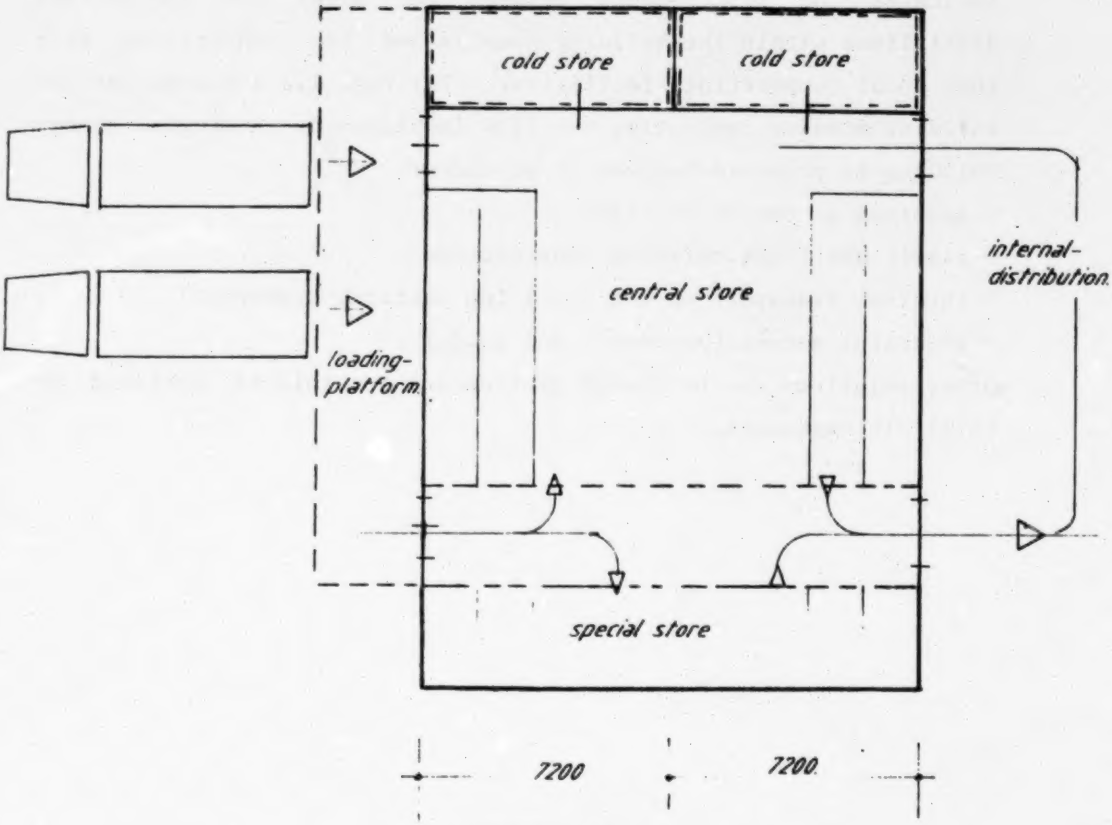
These flows require a good internal road system between the different buildings and a good external road network for central transport in and out with facilities for loading and unloading and parking. The specific flow pattern of the vaccine is laid down in the production units.

4.2.3 The design

The structural design describes the area to be built and indicates the relationship between the areas for the various disciplines within the building complex and the connections with the local supporting facilities. In Fig. 4.2 a scheme for the building modules indicating the flow is proposed. A single storey building is proposed because it provides:

- generous access of daylight
- simple and light building construction
- internal transport on the level (no vertical transport)
- plentiful access (personnel and goods)

Other solutions to the design problem are possible if dictated by local circumstances.



CENTRAL STORAGE

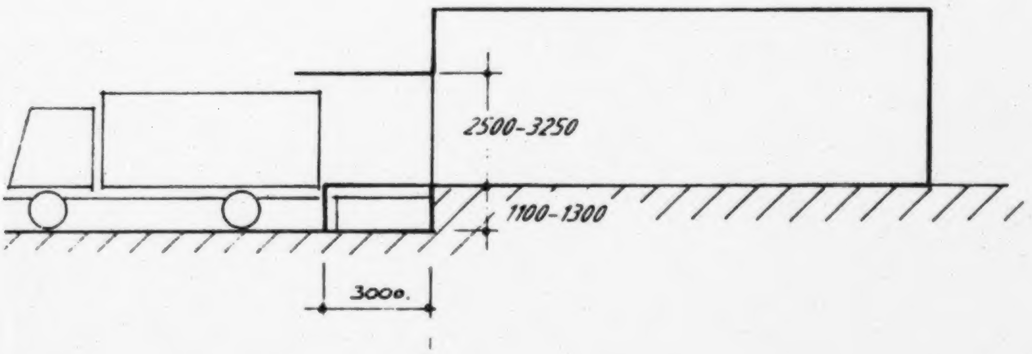


Fig.- 4.3-

4.3 Specification of main functions

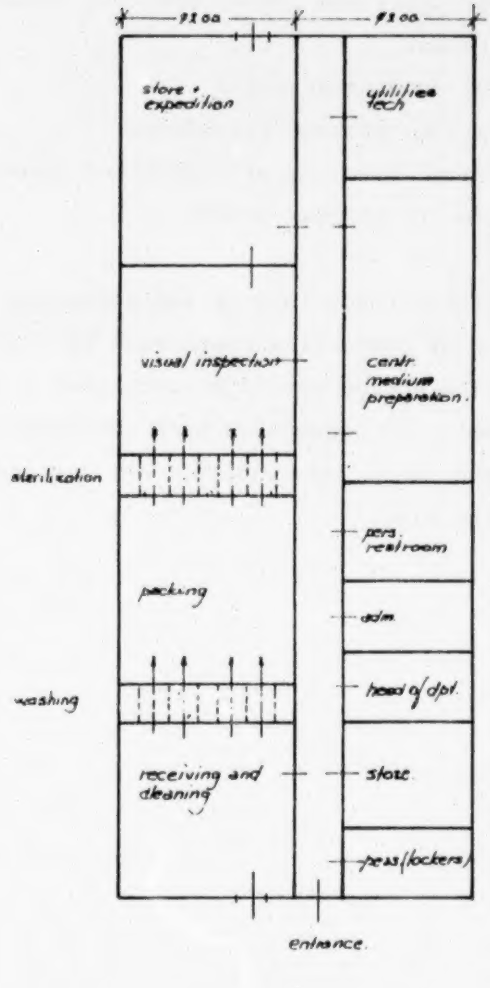
In the previous chapter (4.2.1) the plan is subdivided into 8 different main functions which are shown and defined in the design diagram (fig. -4.2-).

4.3.1 External delivery system and storage (fig. -4.3-)

A central delivery and store for the whole complex offers important advantages:

- maximum control and registration
- optimal appropriate storage facilities
- good stock-control by means of controlled issue
- cost staving due to minimum stocks

A good delivery road, loading and unloading facilities and an outgoing network of connecting roads must be organized for internal provisioning. The design should be such that a large diversity of space is possbile in connection with the diversity of goods which have to be stored, such as cooled, not cooled, chemicals, raw materials and the like.



central kitchen

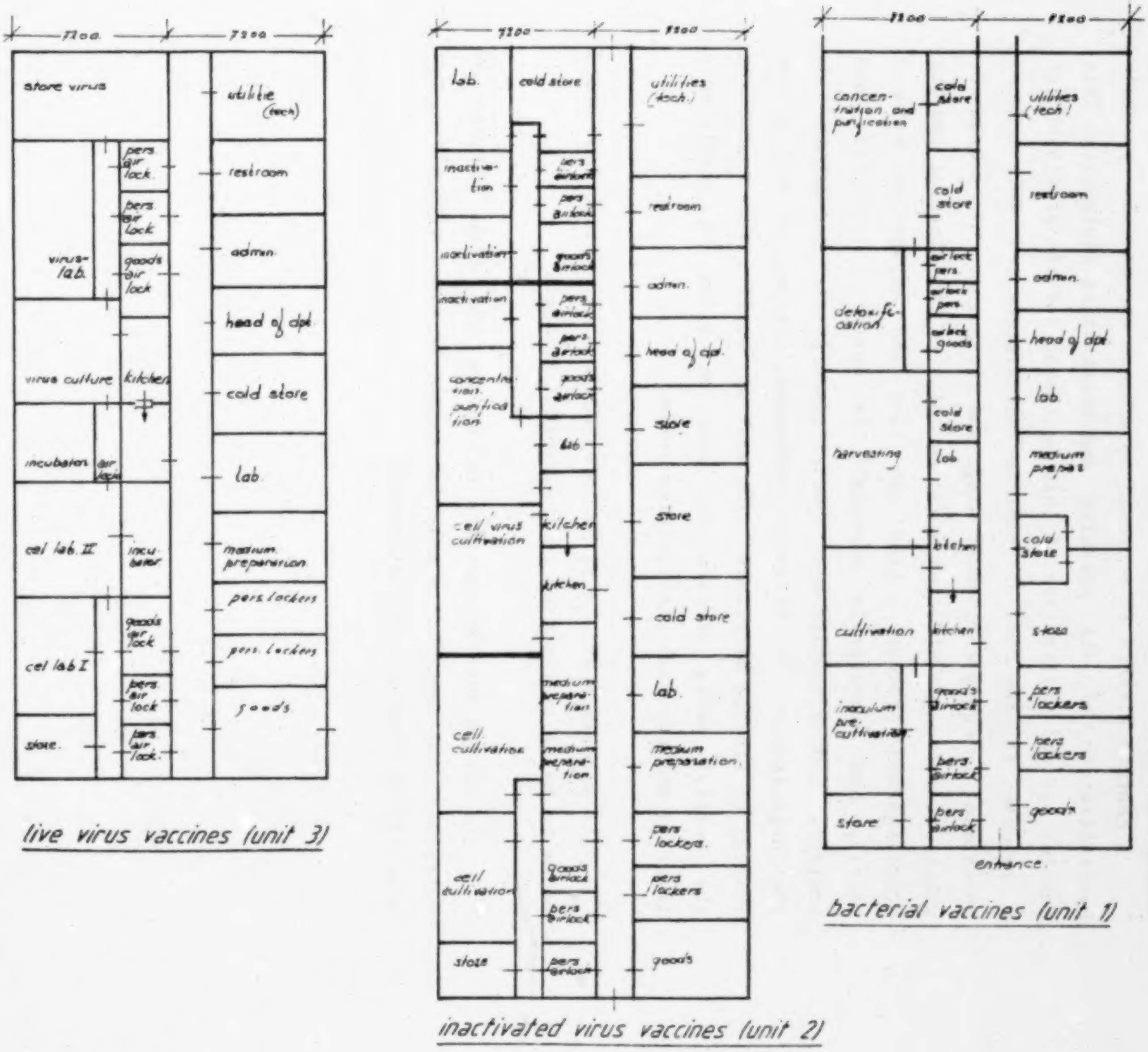
Fig.-4.4-

4.3.2 Central kitchen (fig. -4.4-)

A central kitchen is proposed to achieve the maximum possible centralization of all cleaning and sterilizing equipment. This enables optimal utilization of this equipment and the provision of a reserve in the case of problems and maintenance. This reduces investment in equipment, power usage and the number of personnel, while it increases efficiency and security of the cleaning and the sterilization process. Also the flow and separation between sterile and non-sterile materials is easier to simplify and control. Centralization does however place greater requirements on the organisation of delivery, packaging, storage, as well as the removal of sterile materials. The wash up facility also containing the central media preparation, must be sited to enable easy internal and external distribution roads.

All materials should be disinfected in the production units prior to being transported to the central kitchen.

The design must be executed to ensure the flow pattern of materials so that dirty material cannot be mixed with clean, nor non sterile with sterile equipment.



live virus vaccines (unit 3)

inactivated virus vaccines (unit 2)

bacterial vaccines (unit 1)

Fig.-4.5-

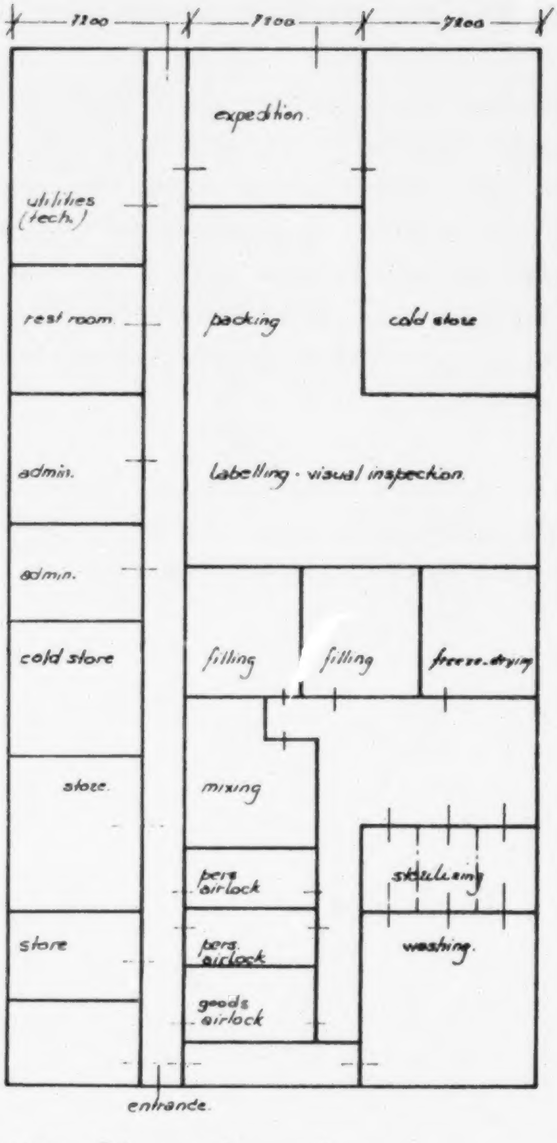
4.3.3 Production units (fig. -4.5-)

There are 3 parallel vaccine production units working on a time sharing (campaign) basis:

- unit 1, intended for: tetanus, diphtheria, BCG and pertussis
- unit 2, intended for: killed polio vaccine type 1, 2 and 3, and rabies
- unit 3, intended for: live attenuated measles

Each of these units has very specific closed work conditions and a logical succession of general, cultivation, processing, inactivation and storage areas with the appropriate support facilities. Personnel and goods enter the specific production areas via airlocks, and to gain access to some areas a second series of airlocks is needed. Where necessary, areas are included for disinfecting materials before removal to the central washing up kitchen or of waste.

For the technical installations (ventilation, electrical power, gases, water and sanitary) sufficient space must be reserved in the roof construction, raised ceilings and space under the floors.



Mixing - filling and freeze-drying.

Fig.-4.6-

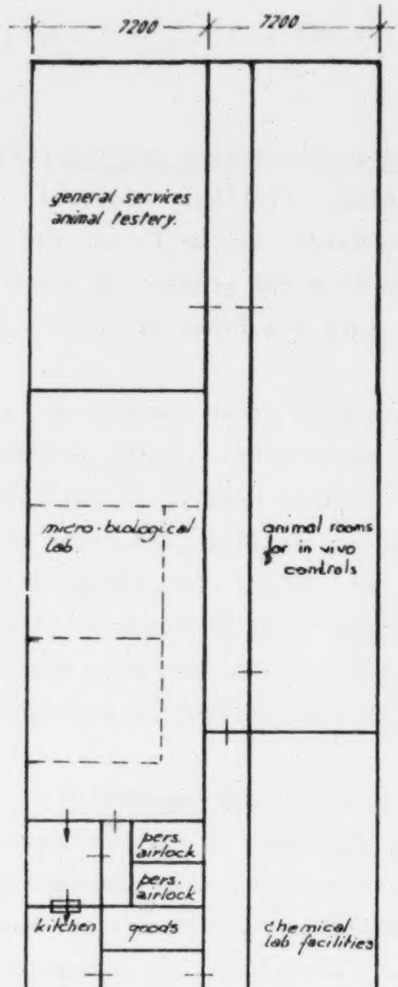
4.3.4 Mixing-, filling- and freeze-drying station (fig. -4.6-)

Here the blending, filling, control and freeze-drying functions are accomodated as well as the supporting services. Material is delivered from the production untis and central kitchen for final completion into the final product.

These are ultra clean areas requiring a high standard of finish and air management. Some products are mixed and subsequently filled, quality controlled and labelled, whilst others are freeze-dried after filling and then quality controlled and labelled. Finally, are filled or freeze-dried products always packaged before storage - our practice is to store filled vaccines in special labelled boxes. They are only packed when all tests are completed and dispatch to distribution is required.

Storage of finished products and expedition

The end product is stored in the warehouses and cold stores intended for the final storage, mass packaged and ready for sending out. The loading/unloading facilitites for expedition are the same as those at central reception. The situation of these warehouses is strongly determined by the local infrastructure as well as the internal routing.



control laboratory

Fig.-6.7-

4.3.5 Control laboratories (fig. -4.7-)

The main functions within the control laboratories are:

- biochemical laboratory
- bacteriological laboratory
- virological laboratory
- animal house

The virological and microbiological laboratory should both be provided with a separate entrance airlock, as well as disinfecting facilities. All sections of the laboratory have a close relation with the production units and the mixing, filling and freeze-drying department.

4.3.6 Support services

This covers, besides the laboratory departments, all control functions such as management, administration, civil and technical services. These functions are related to the other parts of the building and do not have to be sited in a particular position within the complex. It is proposed that all civil functions should also be within the building complex area, for example, the staff canteen, as well as a laundry for the industrial clothing.

4.4 Cost estimation

In view of the unknown local circumstances in the host country, it is impossible to provide an exact cost estimate on the basis of a price per m^2.

However, an estimate of the price per m^2 of the building complex described in this report according to the Dutch experience for 1985 can be given at c. f. 5,000,--. The estimated total costs for 6,000 m^2 would thus amount to f. 30,000,000,-- exclusive of the required furnishings.

APPENDIX

I Air conditioning installation

II Steam and hot water

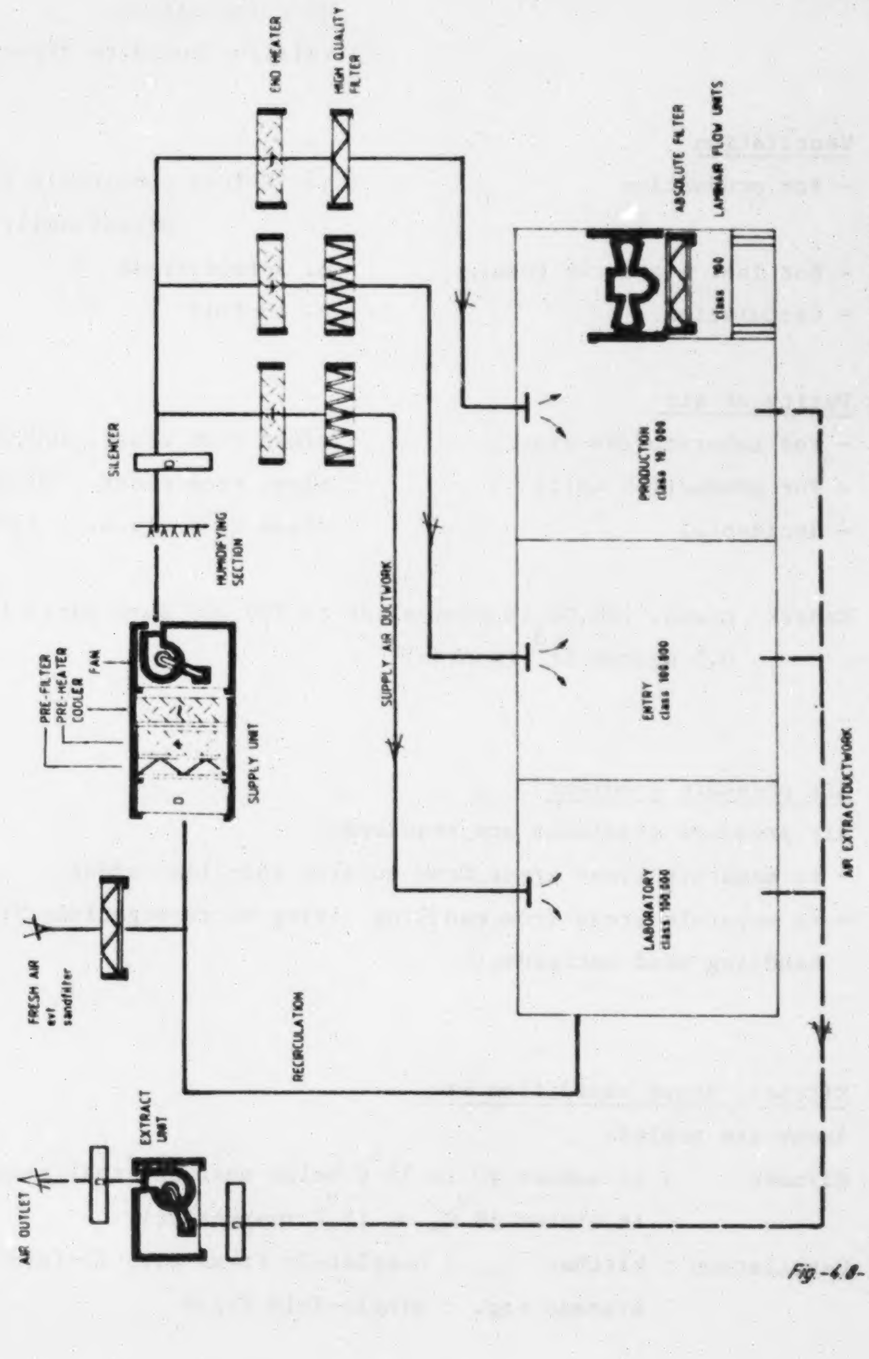
III Power plant

IV Cooling

V Water treatment

VI Gas installations

VII Sewage system



air conditioning installation

Fig.-4.8-

I CLIMATE (design criteria) (fig. -4.8-)

Production buildings

Design room temperature : 18°C for work areas
20°C for offices
relative humidity 35%-65%

Ventilation

- For production : c. 10-fold completely fresh occasionally higher
- For lab. + general room : c. 3-fold fresh
- Circulation : c. 10-fold

Purity of air

- For laboratories etc. : clean room class. 100,000
- For production units : clean room class. 10,000
- Incidental : clean room class. 1,000

Remark: class. 100,00 is equivalent to 100,000 dust particles of 0.5 micron/ft^3 (Ashrae)

Air pressure gradient

Air pressure gradients are required:

- to separate clean areas from outside non-clean areas
- to separate areas from handling living micro-organisms from those handling dead antigens

Kitchen, store expedition etc.

Areas are sealed.

Climate : in summer 10 to 15°C below max. external temperature
in winter 18°C, or 15°C respectively

Ventilation : kitchen : completely fresh air, 20-fold
storage etc. : single-fold fresh

Planned installation

Production buildings

Air conditioning installation, consisting of:

- sand filters (labyrinth)
- pre-filters
- recycling sections
- heaters/coolers
- humidifying sections
- fans
- end-filters (HEPA)
- silencers
- air-ducts

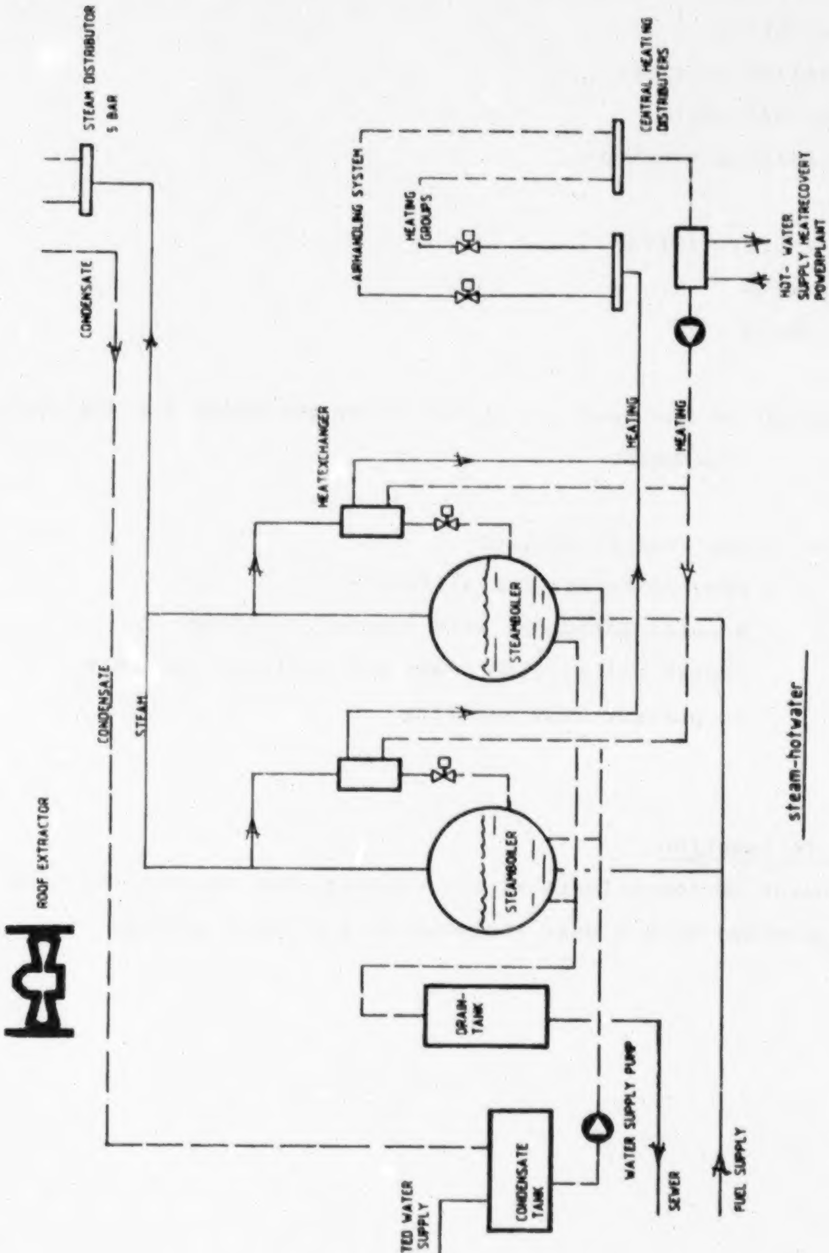
Situation: on the roof and in the rooms set aside for engineers' equipment

Remark: clean room conditions:

- provide rooms with airlocks
- provide personnel with special clothing
- finish walls, floors and all surfaces smoothly to prevent dust settling

Heat reclamation

Dependent on the climate of the country, the air conditioning systems are provided with a heat reclamation e.g. heat system.



steam-hotwater

Fig.-6.9-

II STEAM AND HOT WATER (fig. -4.9-)

To be fitted in the central boiler house:

The steamboiler in order to provide steam for humidifying, sterilisation and kitchen use.

Heating by means of heat exchangers.

Boilers are fuelled by means of the most economic fuel, usually gas or oil.

Steam pressure c. 5 bar, for use in the kitchen c. 2 bar and sterilisation c. 4 bar.

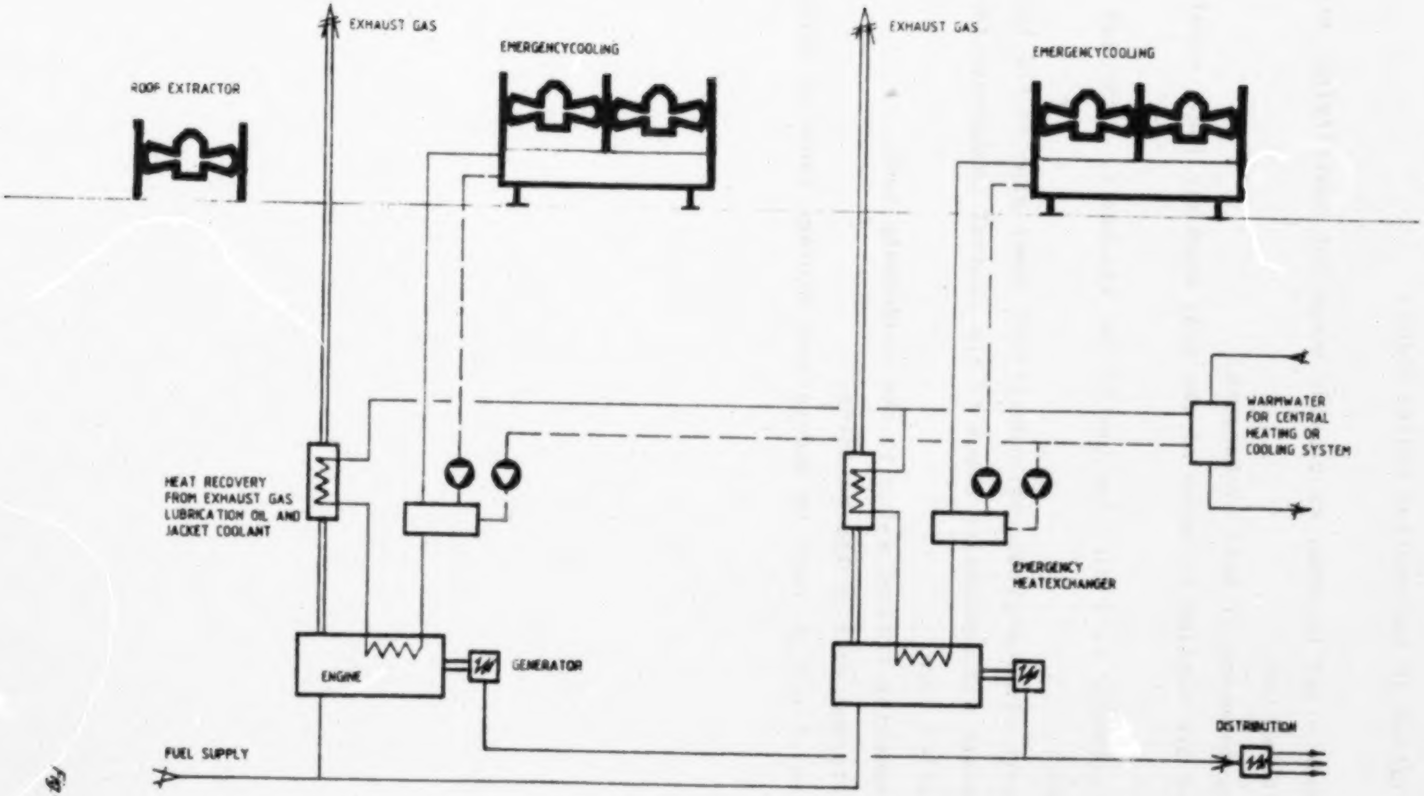
Transport via overground or underground steel pipe to the buildings.

Condensate via condensate pipes to the central condensate tank in the boiler room.

Supplementing treated water in the condensate tank.

Fuel-storage outside the building.

In case of oil in over- or underground storage tanks as appropriate.



powerplant

Fig.-4.10-

III POWER PLANT (fig. -4.10-)

To be fitted in the power plant:

The energy generator unit for the supply of electricity of the complex. Dependent on circumstances as:
- total energy isntallation or as
- emergency power unit in parallel to the mains

Total energy

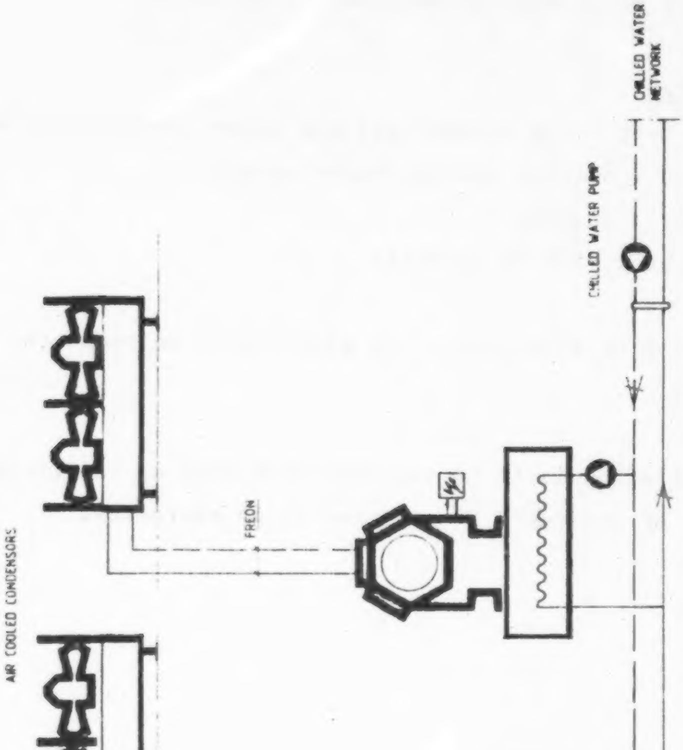
Recovered heat (from exhaust gas and jacket coolant) to be used for:
- in winter : heating and hot water supply
- in summer : cooling

Total output c. 80% of capacity

Distribution of electricity via electricity mains to the various buildings.

The installation is to be supplied with back up facilities so that power can be generated at any time in an emergency.

Fuel: oil
gas



central cooling

Fig.-4.11-

IV COOLING (fig. -4.11-)

The decentralised cooling to be installed in the buildings (refrigerators etc.).
The refrigerators to be supplied with their own cooling installations.
The condensor heat to be extracted by air-cooled condensor fitted directly on the roof.

The central cooling
To be installed in the power plant:
- the compressor cooling aggregates are to be electrically driven and the condensor heat is to be extracted by air-cooled condensors.

Remark: in a very sandy location special attention should be paid to air-intake for the condensors (labyrinth)

The distribution of chilled water via a steel network, over- or underground, to the various air conditioning units.

When adopting a total energy installation an absorption cooling machine should also be installed to use the waste heat.

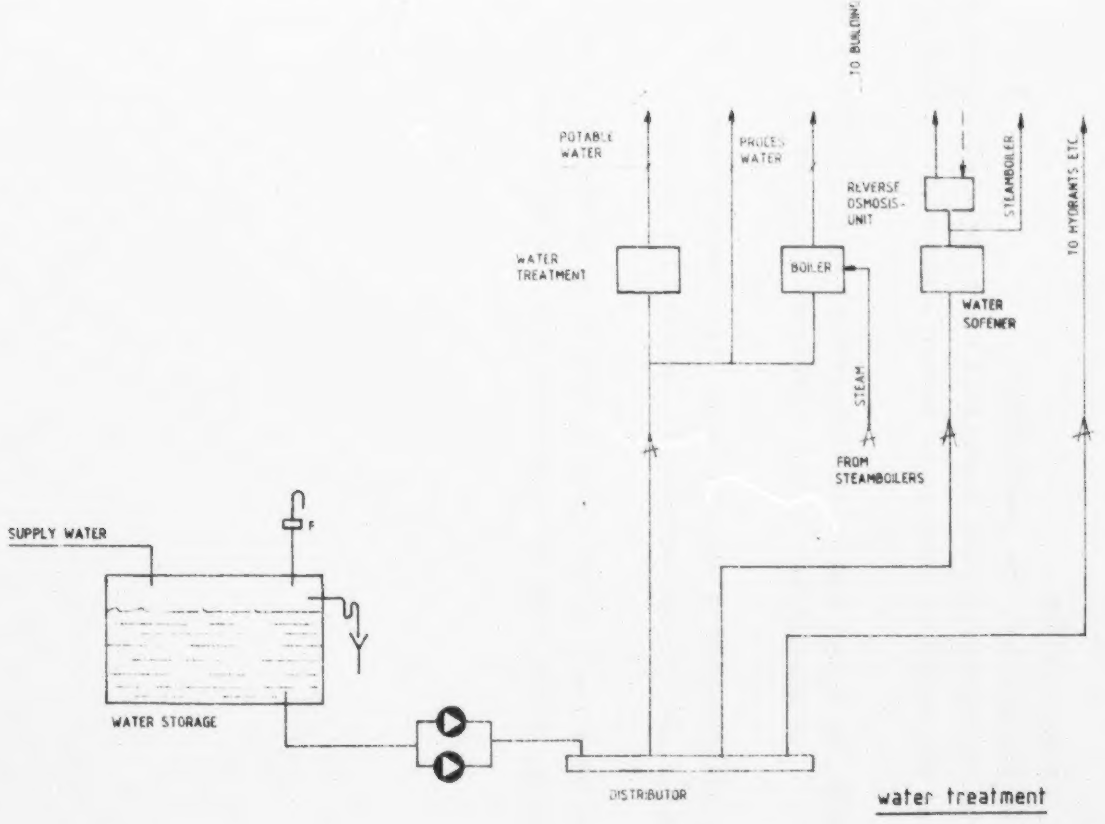

TO BUILDING
POTABLE WATER
PROCES WATER
REVERSE OSMOSIS-UNIT
STEAMBOILER
TO HYDRANTS ETC.
WATER TREATMENT
BOILER
WATER SOFENER
STEAM
FROM STEAMBOILERS
SUPPLY WATER
F
WATER STORAGE
DISTRIBUTOR
water treatment

Fig.-4.12-

V WATER TREATMENT (fig. -4.12-)

The supply of primary water is collected in a water storage tank. It is then transported by means of pumps to the various consumers. The method of water treatment depends on the intended use.

These can be classified as follows:

1. Untreated water
   Required for production of potable water, sanitary appliances and fire hydrants, etc.

2. Softened water
   Required as feed water for steam boilers, various appliances, reverse osmosis- and/or distillation units

3. High quality water
   Required for specific processes (cell cultures) and for finished products (prepared via distillation or reversed osmosis)

Ad. 1

Water supply for drinking has to be of potable quality and purity. Dependent on the quality of the primary water the type of treatment to be applied will vary.
Primary water can be used for the requirements of process water.

For the purposes of hot water production steam-fed boilers are used, which heat the in principal treated water to the required temperature.

Hydrants etc. can be supplied with untreated primary water.

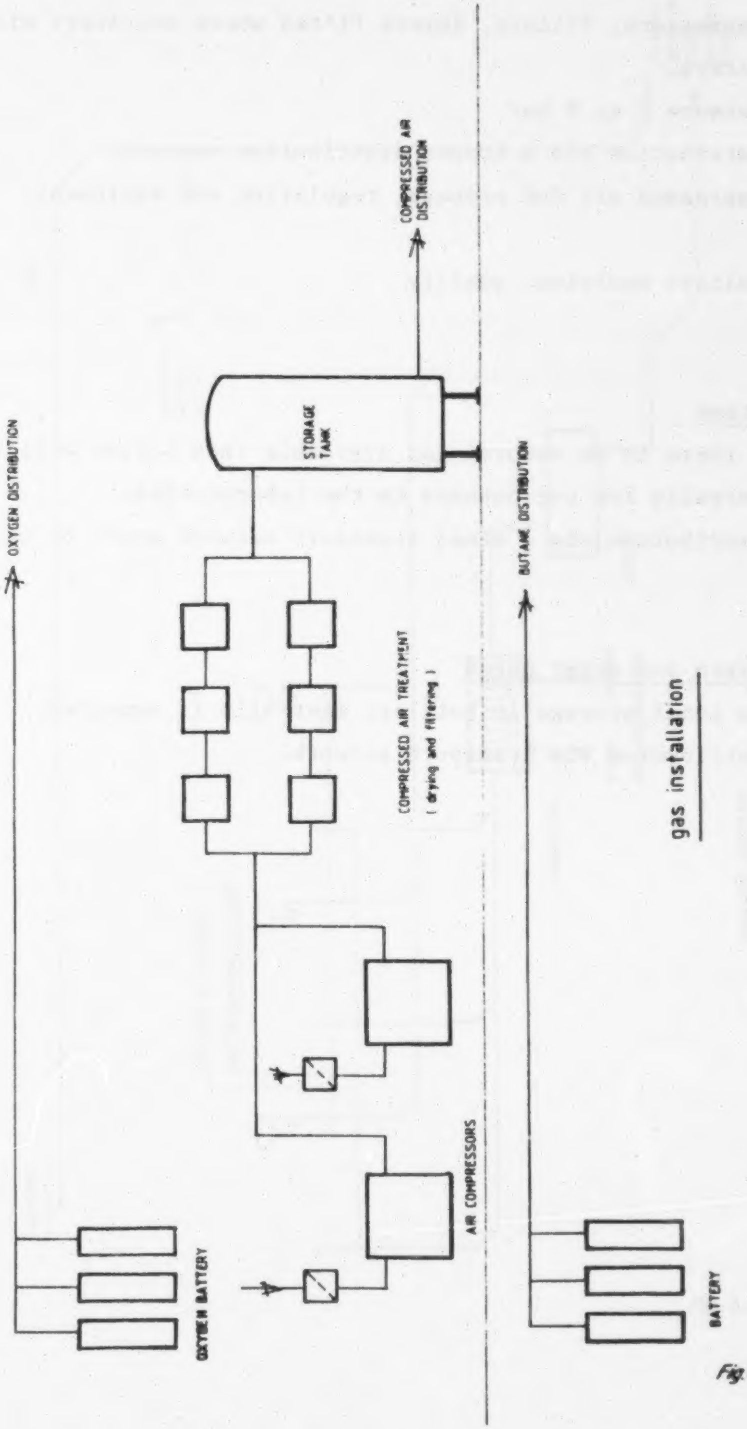
Ad. 2

The required installation consists of the following main components:
- a crushing tank with pressure increasing pump
- the reversed osmosis unit

- the required appliances for disinfection of equipment and distribution network (ultraviolet ray equipment, formalin disinfection equipment)
- the required regulation equipment and safety devices (automatic change-over on breakdown of time switch, conductivity meter etc.)

Ad. 3

Distribution of the osmosis water takes place via a synthetic network, which is fitted circulating to the equipment (fermenting/preparation boilers).



gas installation

Fig.4.13-

VI GAS INSTALLATIONS (fig. -4.13-)

Compressed air

A central compressed air unit housed in the power plant consists of compressors, filters, dryers fitted where necessary with bacteriaproof filters.

Pressure : c. 8 bar

Distribution via a copper distribution network.

Compressed air for process, regulation and equipment.

Quality: medicinal quality

Butane

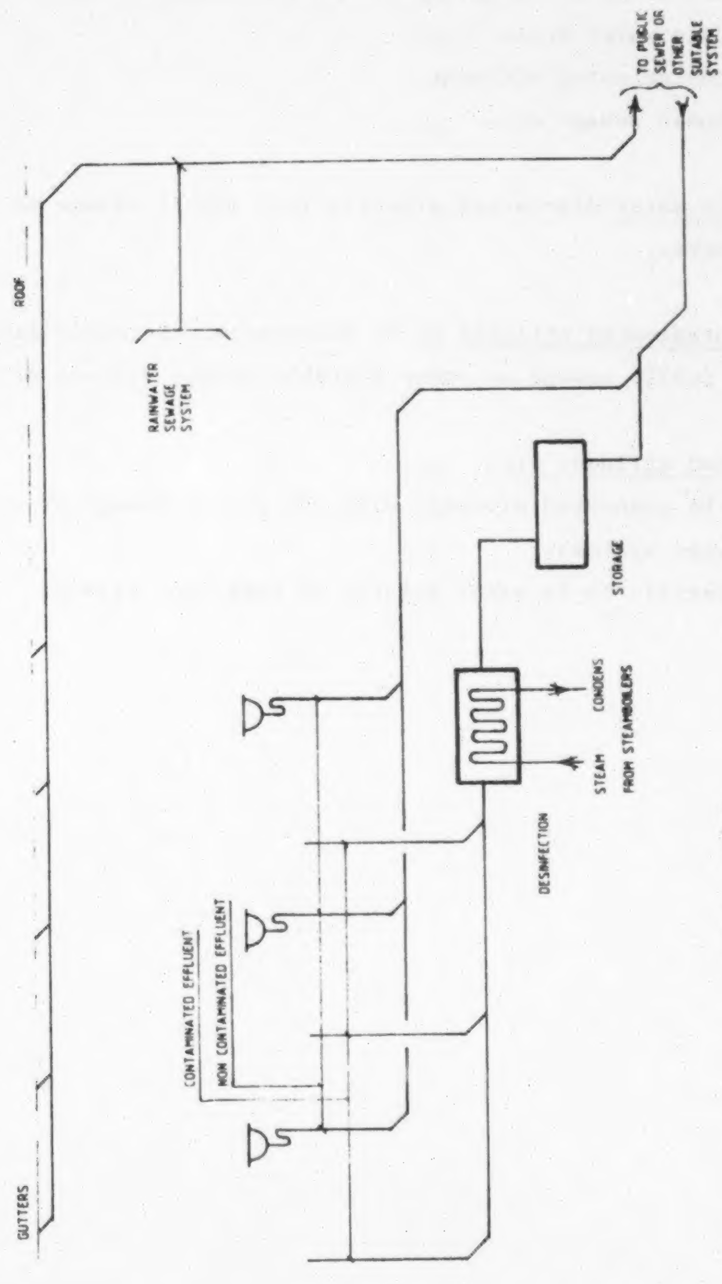
If there is no natural gas available then butane will be placed centrally for the burners in the laboratories.

Distribution via a steel transport network over- or underground.

Oxygen and other gases

Via local storage in bottles; centrally if required.

Distribution via transport network.



sewage system

Fig.-4.16-

VII SEWAGE SYSTEM (fig. -4.14-)

Three sorts of sewage systems are planned, namely:

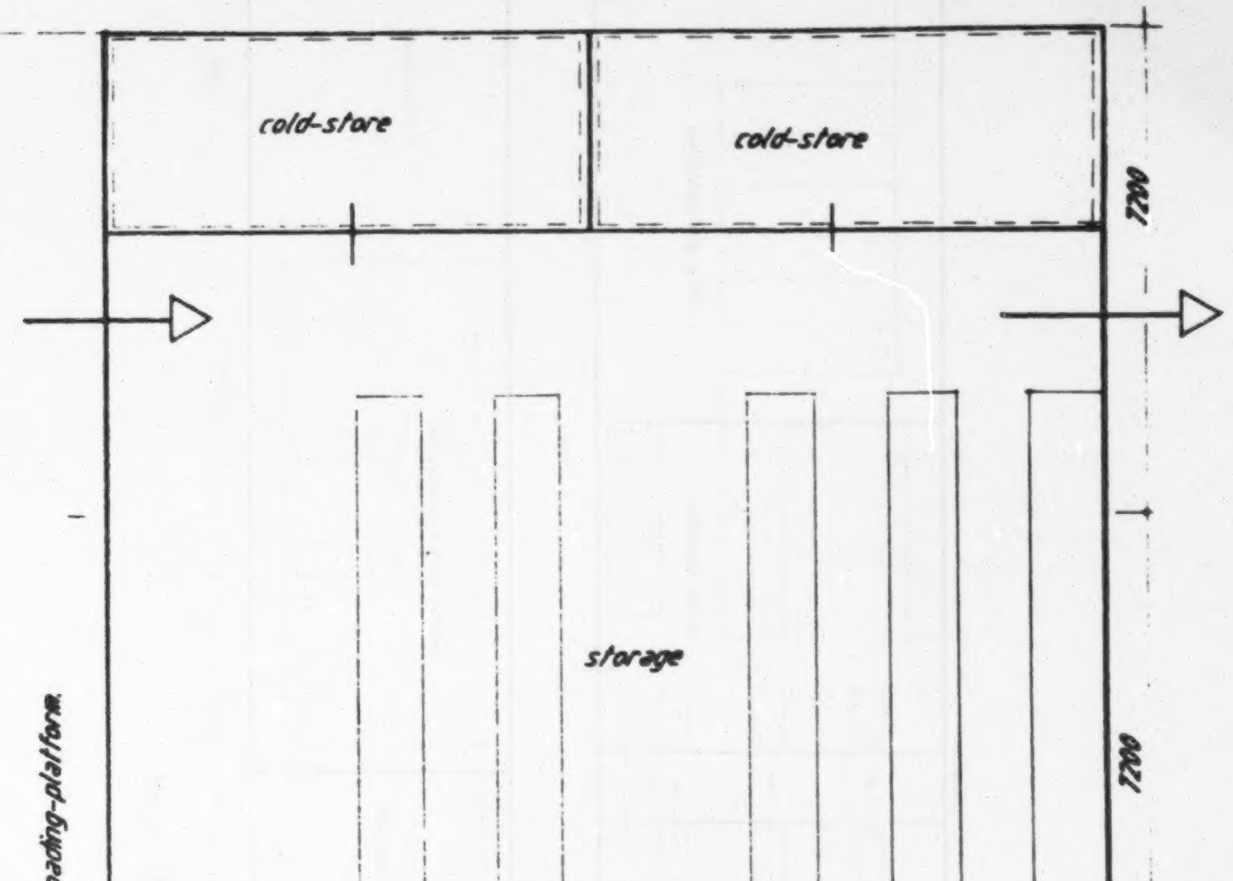
- rain water drain
- contaminated effluent
- human sewage etc.

Rain water discharged directly into public sewage or other sewage systems.

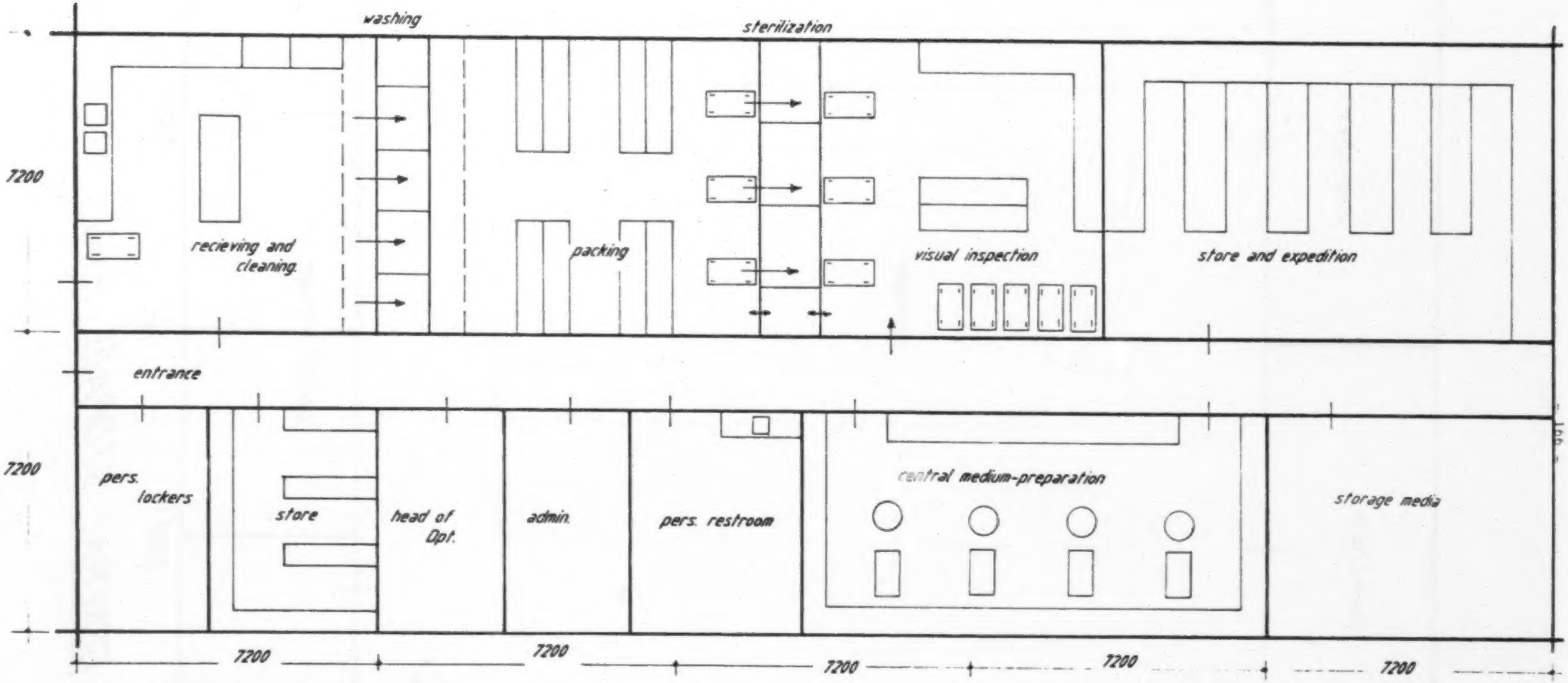
Contaminated effluent to be disinfected thermally first and drained to public sewage or other suitable sewage systems after cooling.

Human effluent etc.

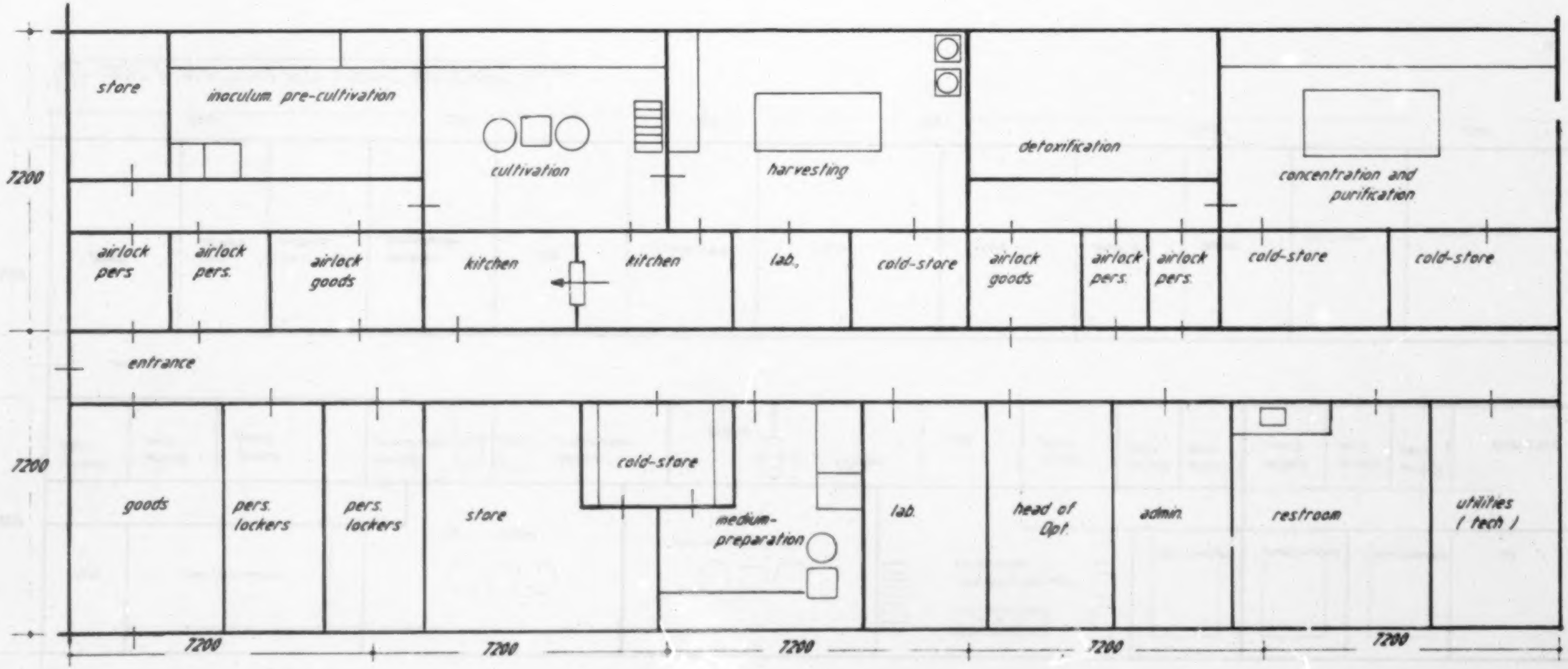
To be connected directly with the public sewage or other suitable sewage systems.
Materials to be used: plastic or cast iron piping.



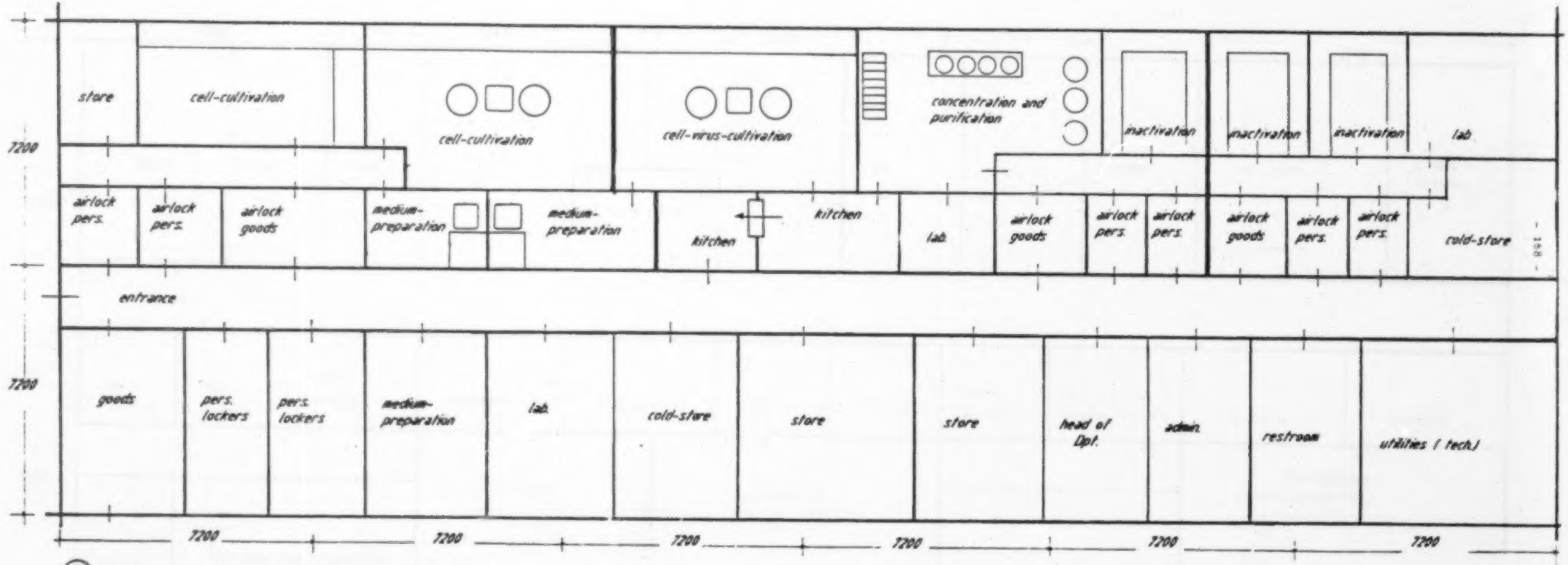
① GENERAL STORAGE (1:100)



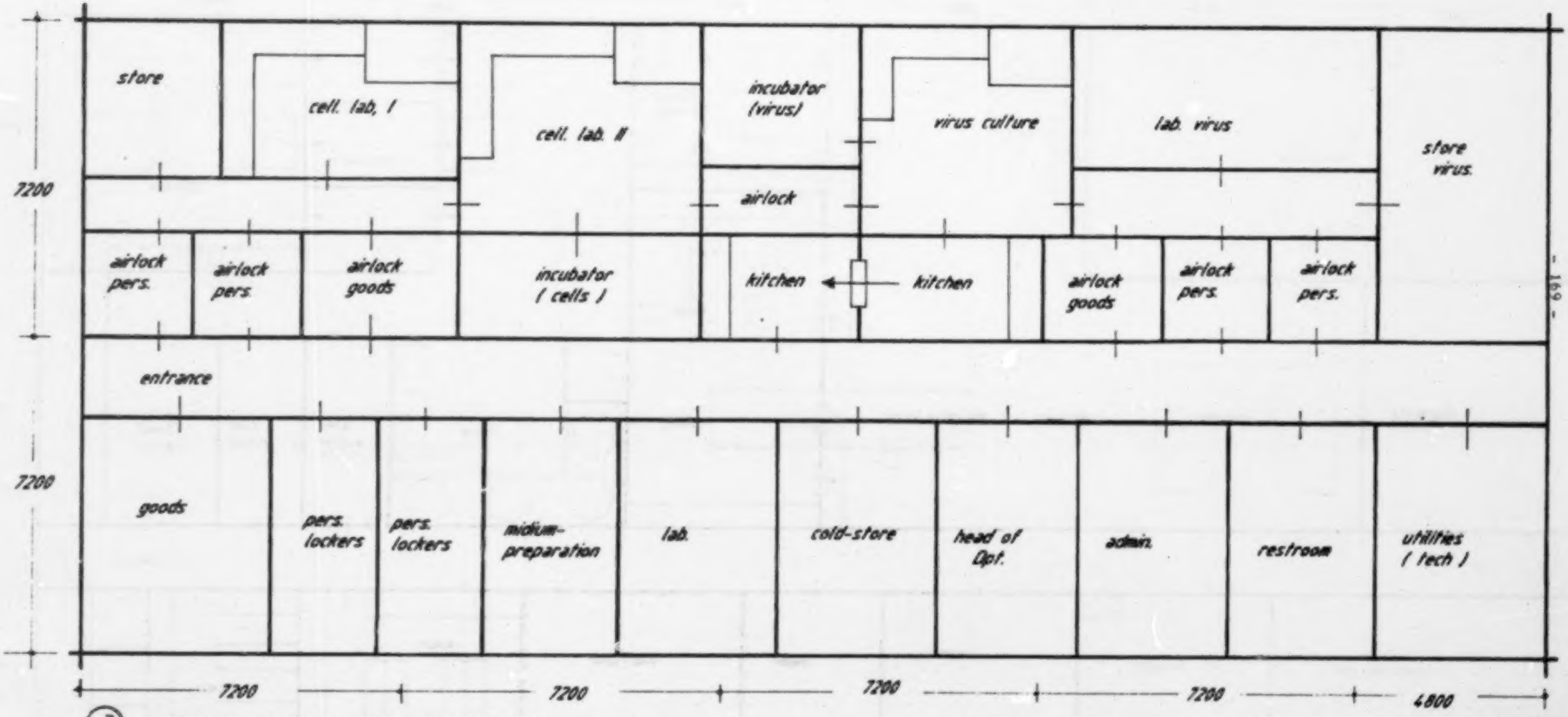
② CENTRAL KITCHEN AND MEDIUM PREPARATION (1:100)



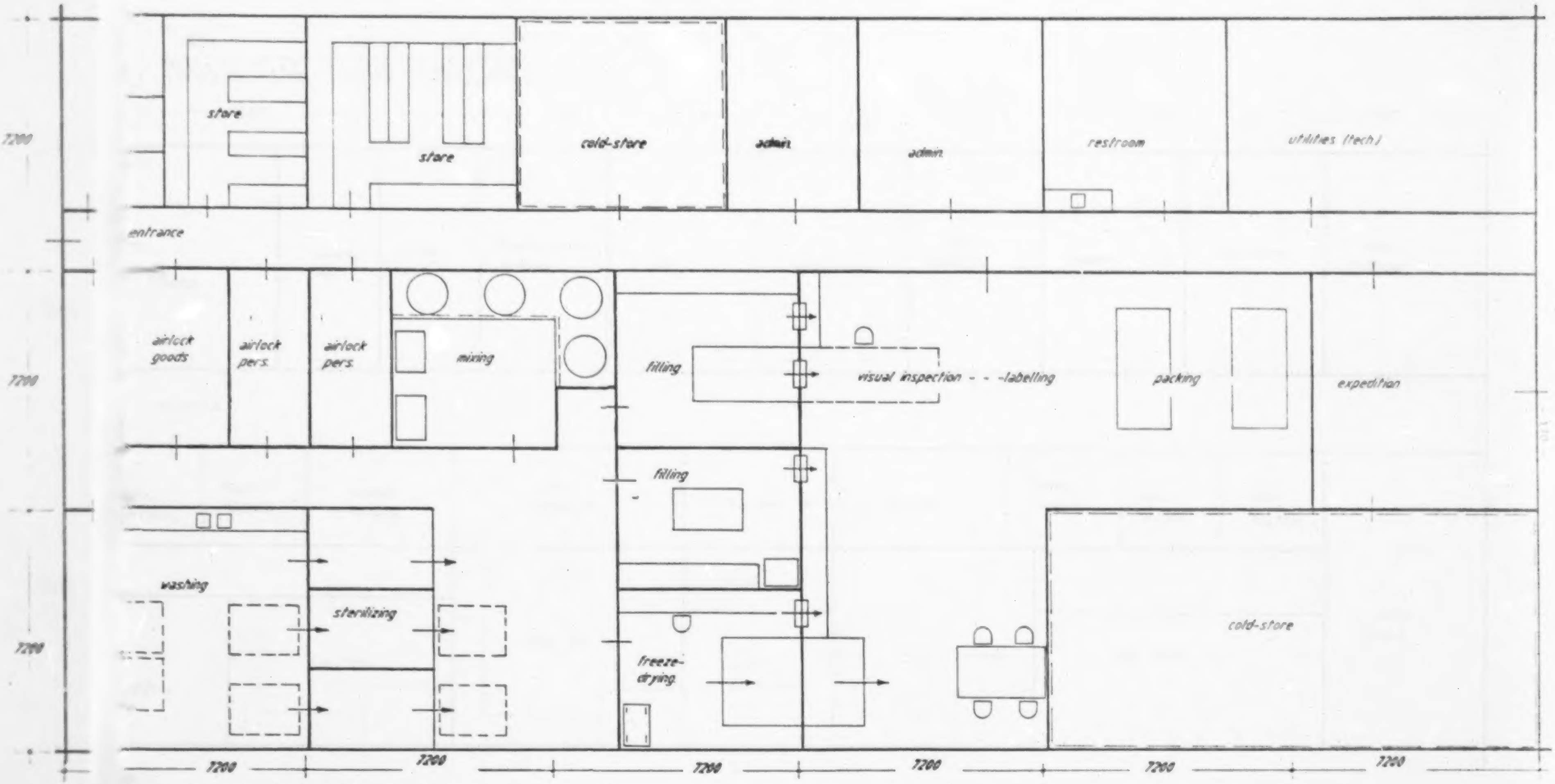
③ UNIT 1 BACTERIAL VACCINES (1:100)



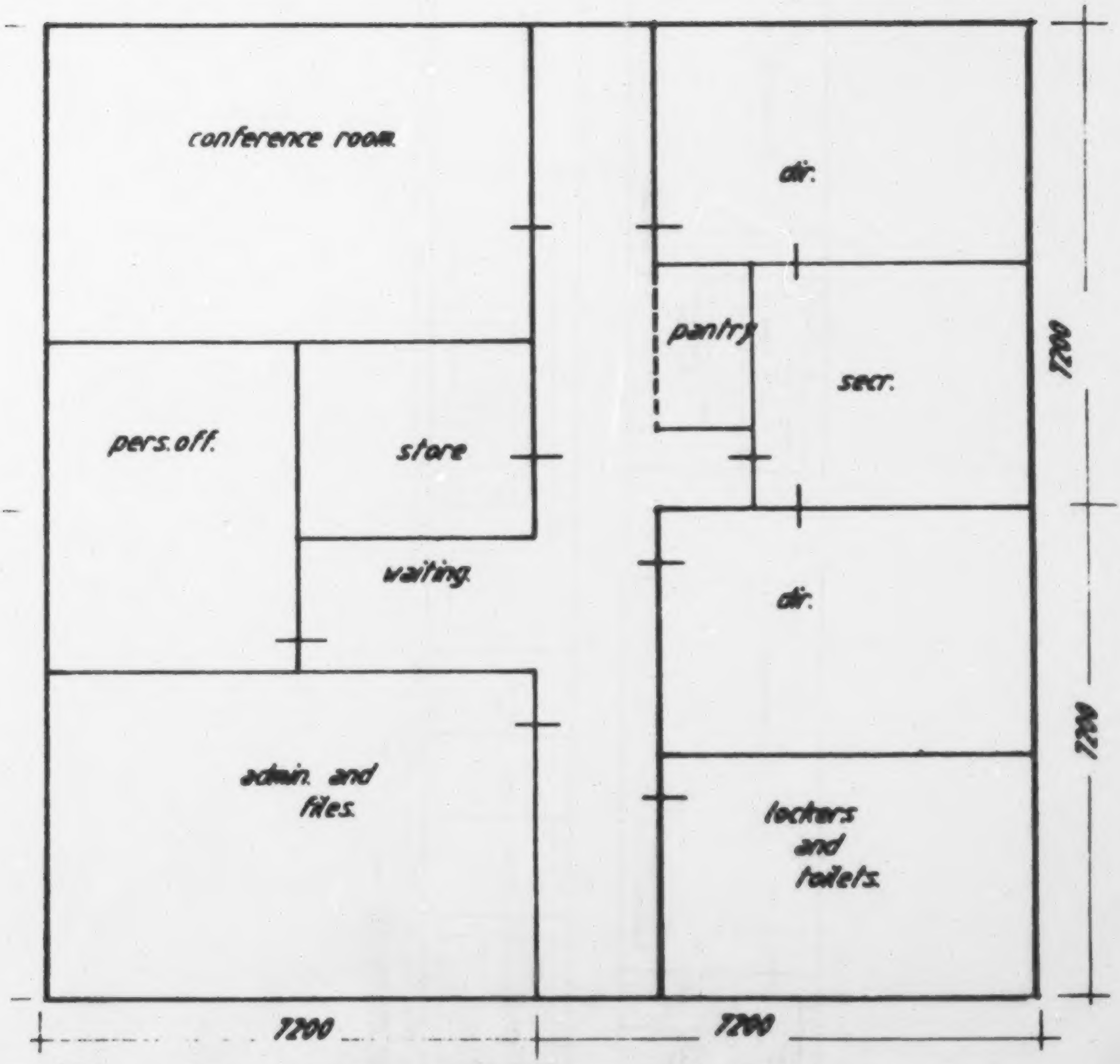
③ UNIT.2 INACTIVATED VIRUS VACCINES. (1:100)



③ UNIT.3 LIVE VIRUS VACCINES (1:100)



④ ING, FILLING AND FREEZE-DRYING. (1:100)


conference room.
dir.
pantry
secr.
pers.off.
store
waiting.
dir.
admin. and files.
lockers and toilets.
7200
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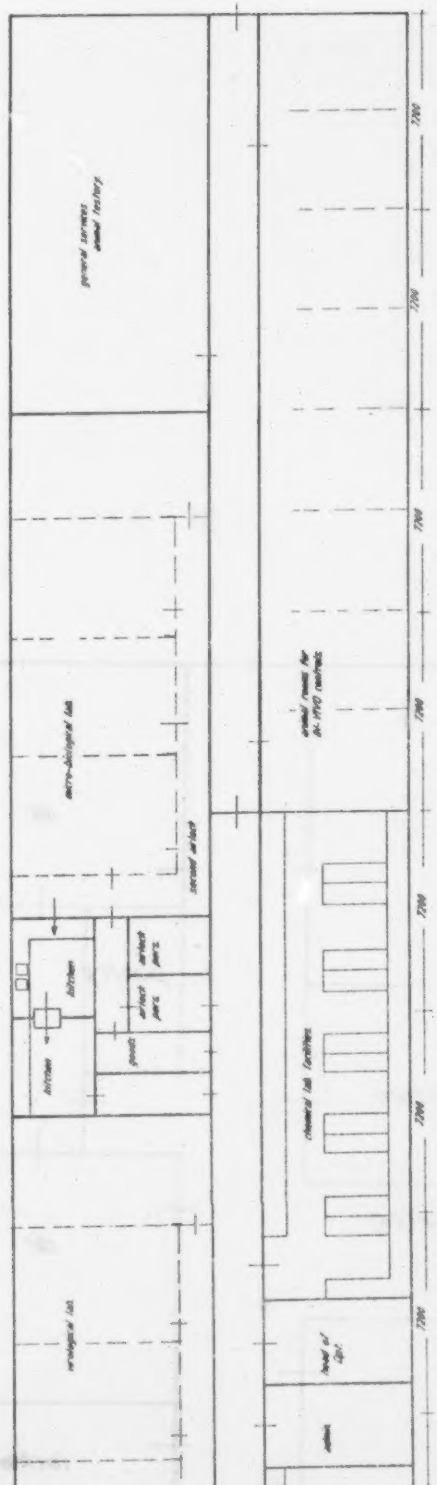
[illegible] MANAGEMENT



ABORATORY FACILITIES (1:100)

5. STAFF TRAINING IN PRODUCTION AND CONTROL

Training of staff is a key element in the transfer of vaccine technology. RIVM has established courses in Production techniques and Quality Control methods for vaccines. These courses are now run under WHO auspices and comprise a four-week intensive course. They are run each year alternately for Production and Control. Staff attending these courses usually come from established laboratories.

The requirements for training staff involved in technology transfer are different. The key elements are set out below:

1. Training should only be started when building of all the local facilities is advanced so that they are ready on the return of the trainees.
2. At least two production and two control employees should be trained simultaneously in order to ensure, as far as possible, that there is a successful acquisition of knowledge and experience. Such training will take at least 3 to 6 months depending on the type of vaccine.
3. Both the production and control staff members should be trained simultaneously at the same host laboratory in order to ensure a uniform approach to problems.
4. At the start of the production and control of all vaccines, it is advisable that collaborators from the host laboratory, fully experienced in production and control, are involved on site locally in the transfer of technology to advise on the training and start-up at the recipient laboratory.
5. The recipient and host laboratory should have a follow-up contact on the technology transfer until all procedures of production and control are running smoothly. This includes exchange of samples of vaccines between both laboratories for testing and regular visits of staff members and vice versa. Some special tests may be carried out in the host laboratory for a period, e.g. tests for residual DNA in vaccines made in VERO cells.

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